A pilot surgical trial to evaluate early immunologic pharmacodynamic parameters for the PD-1 checkpoint inhibitor, pembrolizumab (MK-3475), in patients with surgically accessible recurrent/progressive glioblastoma

Protocol Version Date:	November 13, 2021
Protocol Version No.:	6.0
DF/HCC Protocol No.:	16-225
Merck Protocol No.:	3475-476
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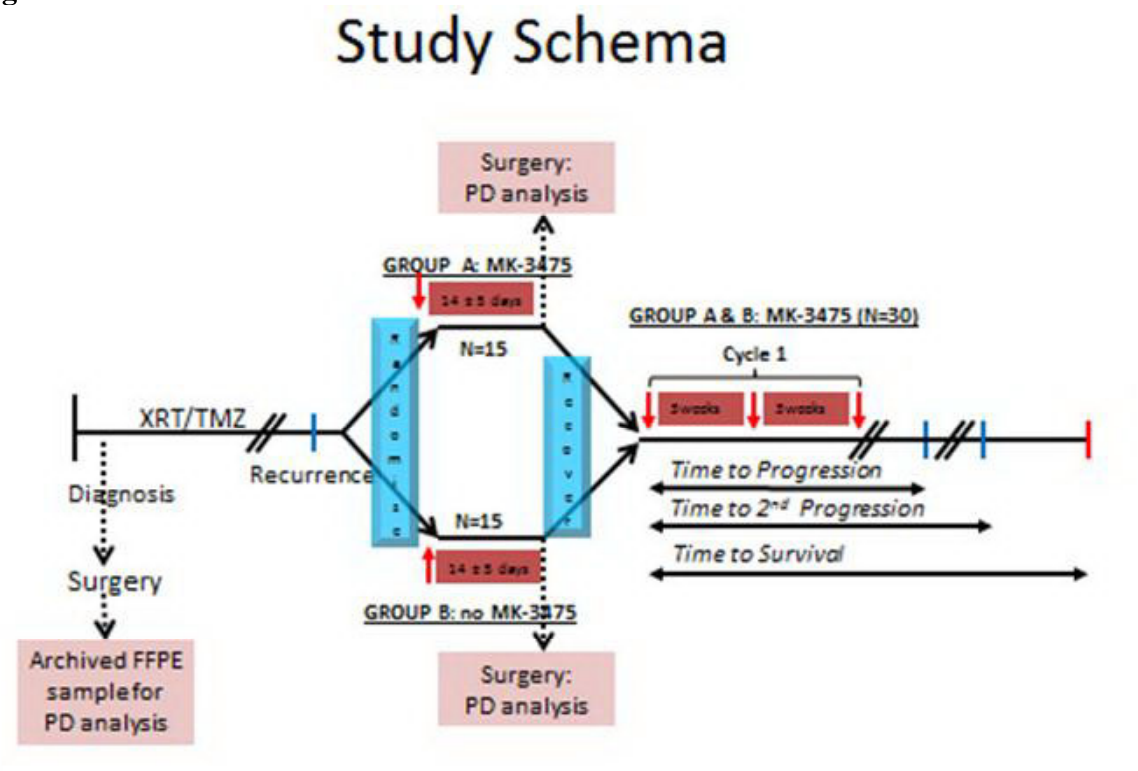
Agent(s):	**Pembrolizumab (**MK-3475), supplied by Merck & Co., Inc.
IND#:	130065
IND holder:	Patrick Y. Wen, MD
Funding/Contractual Sponsor:	Dana-Farber/Partners CancerCare, Inc.
Funding Support:	Merck & Co., Inc.

TRIAL SUMMARY

Abbreviated Title	Pembrolizumab in Surgically Accessible Recurrent GBM
Trial Phase	Pilot
Clinical Indication	Surgically resectable Recurrent glioblastoma
Trial Type	Interventional
Type of control	Presurgery
Route of administration	Intravenous
Trial Blinding	Unblinded, open-label
Treatment Groups	Pembrolizumab presurgery at 200mg followed by Pembrolizumab at 200 mg every 3 weeks post surgery (Group A). No therapy presurgery followed by pembrolizumab at 200 mg every 3 weeks post surgery (Group B)
Number of trial subjects	Approximately 30 evaluable patients will be enrolled in **Stage 1** of the trial, an additional 20 evaluable patients will be enrolled in **Stage 2**.
Estimated duration of trial	The sponsor estimates that the trial will require an additional 18 months to complete **Stage 2** enrollment.
Duration of Participation	Each subject will participate in the trial from the time the subject signs the Informed Consent Form (ICF) through the final protocol-specified contact. In the first stage of the trial (**Stage 1**), after a screening phase of 14 days, eligible subjects will be randomized into two groups (Group A and Group B). In the second stage of the trial (**Stage 2**), an additional 25 patients will be screened for a goal of 20 evaluable patients to be assigned to Group A only. Group A will receive one dose of pembrolizumab at 200 mg within 14± 5 days prior to surgery. Group A patients will receive Pembrolizumab at 200 mg every three weeks after surgery. Group B patients will not receive pembrolizumab prior to surgery but will receive pembrolizumab at 200 mg every three weeks after surgery. Post-surgically, both groups (A and B) will receive treatment during each 6-week dosing cycle. Treatment with pembrolizumab therapy will continue until 1) documented confirmed disease progression, unacceptable adverse event(s), intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the subject, subject withdraws consent, pregnancy of the subject, noncompliance with trial treatment or procedure requirements; or 2) administrative reasons. After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for up to 90 days after the end of treatment). Subjects who discontinue for reasons other than disease progression will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent, or becoming lost to follow-up. All subjects will be followed by telephone or medical record review for overall survival until death, withdrawal of consent, or the end of the study.

STUDY SCHEMA

Stage 1



Stage 2: up to an additional 25 patients will be enrolled to Group A, with the goal of 20 evaluable patients.

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Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept. 49

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1. OBJECTIVES

1.1 Study Design

This is a multicenter, randomized, open-label, pilot surgical trial of pembrolizumab (MK-3475) among bevacizumab naive, surgically resectable recurrent glioblastoma patients. Please see Figure 1 for Schema.

> **Group A:** pembrolizumab (MK-3475) at 200 mg IV within 14± 5 days prior to surgery. Post-surgery subjects will receive pembrolizumab at 200mg IV every three weeks.
> **Group B:** no pembrolizumab (MK-3475) prior to surgery. Post-surgery subjects will receive pembrolizumab at 200mg IV every three weeks.

After recovering from surgery, subjects will be evaluated every 6 weeks with radiographic imaging to assess response to treatment. The Response Assessment in Neuro-Oncology (RANO) criteria will be used as the efficacy endpoint of response rate. A modified RANO (iRANO)[111] (as described in Section 10.0) will be used to evaluate response and progression in an exploratory fashion due to the tumor response patterns seen with pembrolizumab treatment (e.g., tumor flare). Adverse events will be monitored throughout the trial and graded in severity according to the guidelines outlined in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. Treatment with study therapy will continue until documented disease progression, unacceptable adverse event(s), intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the subject, subject withdraws consent, pregnancy of the subject, noncompliance with trial treatment or procedure requirements, or administrative reasons. After the end of treatment, each subject will be followed for 30 days for adverse event monitoring and 90 days for serious adverse event reporting. Subjects who discontinue treatment for reasons other than disease progression will have post-treatment follow-up of disease status until disease progression, initiation of a non-study cancer treatment, withdrawing consent, or becoming lost to follow -up. All subjects will be followed by telephone contact or medical record review for overall survival until death, withdrawal of consent or the end of the study, whichever comes first.

Our initial hypothesis as reflected in the design of Stage 1 of the trial, is that a functional immune defect exists within the T lymphocyte compartment in recurrent/progressive glioblastoma patients. The neoadjuvant use of pembrolizumab (PD-1 blocking mAb) in this patient population will lead to a statistically significant increase in tumor infiltrating lymphocytes when compared to a concurrent control population. The combination of tumor tissue and optional imaging evaluations should provide insight into immune activation, antitumor effect and toxicity with immune checkpoint inhibitors.

An interim analysis of data from 35 enrolled patients from October 2016 to September 2017 in this trial has also shown that patients who were randomized to receive neoadjuvant pembrolizumab, with continued adjuvant therapy following surgery (Group A), had significantly extended overall survival compared to patients that were randomized to receive adjuvant, post-surgical PD-1 blockade alone (Group B) (hazard ratio = 0.39; P = 0.04, log-rank test). Neoadjuvant PD-1 blockade was associated with upregulation of T cell and interferon-γ-related genes, but

downregulation of cell cycle-related genes within the tumor, which was not seen in patients that received adjuvant therapy alone[112]. Therefore, in a second stage extension of this trial (Stage 2), the specific effect of neoadjuvant pembrolizumab on the cell cycle and cancer proliferation related genetic signature within the tumor microenvironment of progressive/recurrent glioblastoma patients will be evaluated.

Specific procedures to be performed during the trial, as well as their prescribed times and associated visit windows, are outlined in the Study Calendar - Section 9.

1.2 Primary Objectives & Hypothesis

Stage 1:

1.2.1 Objective: To test the hypothesis that administration of pembrolizumab (MK-3475) will induce statistically significant increases in tumor infiltrating T lymphocyte (TIL) density in recurrent/progressive GBM patients compared to an untreated concurrent control versus the null hypothesis of no difference the treatment and control groups. (Group A vs. Group B).

Hypothesis: Our hypothesis is that a functional immune defect exists within the T lymphocyte compartment in recurrent/progressive glioblastoma patients. The neoadjuvant use of pembrolizumab (PD-1 blocking mAb) in this patient population will lead to a statistically significant increase in tumor infiltrating lymphocytes when compared to a concurrent control population.

Stage 2:

1.2.2 Objective: To test the hypothesis that administration of pembrolizumab (MK-3475) will lead to statistically significant changes, specifically decreased expression of the cell cycle/cancer proliferation related genetic signatures, within the tumor mircroenvironment of recurrent/progressive GBM when compared to an untreated concurrent control versus the null hypothesis of no difference the treatment and control groups. (Group A vs. Group B).

Hypothesis: Our hypothesis is that neoadjuvant use of pembrolizumab (MK-3475) will lead to statistically decreased expression in the cell cycle/cancer proliferation related genetic signature as measured by RNA sequencing when compared to a concurrent control population.

For both Stage 1 and Stage 2:

1.2.3 Objective: To evaluate safety of study drug in this patient population.

1.3 Secondary Objective

1.3.1 Objective: To estimate percent PFS6 in this patient population using RANO criteria.

1.4 Exploratory Objectives

1.4.1 Objective: To evaluate the associations between exploratory biomarkers and clinical outcomes and adverse events these include:

1.4.1.1 Estimate correlation of quantitative assessments of TIL density or clonality with clinical responses to pembrolizumab in recurrent glioblastoma patients.

1.4.1.2 Estimate efficacy of pembrolizumab by PFS, second PFS and OS. (Groups A and B) as defined by RANO.

1.4.1.3 Estimate efficacy of pembrolizumab by PFS6, PFS, second PFS and OS. (Groups A and B) as defined by iRANO.

1.4.1.4 Explore effect of pembrolizumab on TIL proliferation (CD8+KI-67+ staining).

1.4.1.5 Estimate difference in PD-1 and PDL-1 IHC expression between Group A and B as well as between archived and study samples.

1.4.1.6 Explore whether oligoclonal T cell populations within tumor tissue are similarly expanded in peripheral blood after pembrolizumab, the magnitude of which correlates with clinical responses.

1.4.1.7 Explore if changes in specific MRI parameters correlate with tumor and peripheral blood immune responses.

1.4.1.8 Correlation of tumor mRNA from extracellular vesicles with outcome.

2. BACKGROUND

2.1 Study Disease

Glioblastoma is the most common primary glial neoplasm of the central nervous system in adults.[1] Despite rigorous therapeutic efforts, overall prognosis remains dismal. Specifically, multimodality therapy integrating surgery, radiation therapy and temozolomide chemotherapy is associated with a median progression-free survival of only 6.9 months, and a median overall survival of 14.6 months.[2] Following progression, salvage therapies have historically been of nominal benefit with PFS-6 rates under 10% noted in recent meta-analyses.[3-5]

2.2 Background: Pembrolizumab

Refer to the Investigator's Brochure for detailed information on pembrolizumab.

2.2.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades.[6] Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies.[7-11] _ENREF_2 _ENREF_2 In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2).[12,13] The structure of murine PD-1 has been resolved.[14] PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade.[12,15-17] The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins.[18,19] _ENREF_13 PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells.[20,21] _ENREF_15 Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells.[22] The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors.[19,23-25] _ENREF_18 Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues.[19] Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL).[26] This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab (previously known as MK3475 and SCH 900475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2.

2.2.2 Preclinical and Clinical Trial Data

Refer to the pembrolizumab Investigator's Brochure for additional Preclinical and Clinical data.

2.2.3 Ongoing Clinical Trials

Ongoing clinical trials are being conducted in advanced melanoma, non-small cell lung cancer, head and neck cancer, urothelial tract cancer, triple negative breast cancer, gastric cancer, and hematologic malignancies. For trial details please refer to the Investigator's Brochure.

2.3 Rationale

2.3.1 Rationale for the Trial and Selected Subject Population

More effective treatments are needed for glioblastoma. Glioblastoma is one of the most lethal of human cancers, with very few long-term survivors and no definitive cures for this disease. These tumors invade and infiltrate the surrounding brain, making complete surgical excision impossible. They are also among the most radiation and chemotherapy resistant cancers, with a median survival of 12-18 months from initial diagnosis, even with surgery, radiation and chemotherapy (1, 2). The glioblastoma patient population has dismal outcomes and innovative approaches are desperately needed.

Figure 1: **PD-1 expression on T cells from glioblastoma patients**

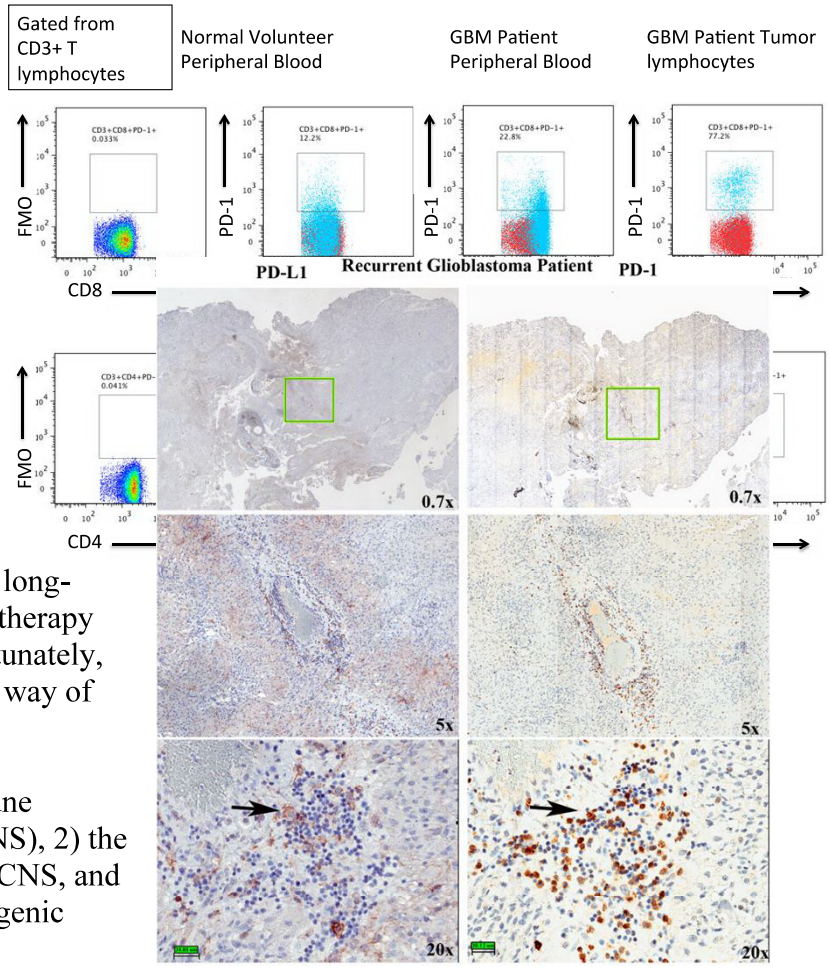

Figure 2: Immunohistochemical staining for PD-1 and PD-L1 in recurrent glioblastoma. Representative PD-1 and PD-L1 IHC staining of a recurrent glioblastoma patient. Staining was performed at Merck Laboratories (FlexTRS97). Note the clear lymphocytic staining of PD-1 and adjacent cellular localization of PD-L1.

Immunotherapy is ideally suited to target isolated pockets of infiltrating tumor cells in the brain. There has been a long-standing interest in applying tumor immunotherapy approaches to primary brain tumors. Unfortunately, these efforts have largely shown little in the way of objective therapeutic efficacy.

Part of the difficulty has been: 1) the "immune privilege" of the central nervous system (CNS), 2) the limited trafficking of immune cells into the CNS, and 3) the lack of well-de fined, highly immunogenic

tumor-rejection antigens (TRA) in gliomas. Our recent pre-clinical studies have demonstrated that we can potentially overcome some of these critical barriers to progress in brain tumor immunotherapy. In a syngeneic, orthotopic glioma mouse model, we demonstrated that PD-1 mAb significantly increased the infiltration of T lymphocytes into murine GL261 gliomas. In addition, peripheral blood and tumor infiltrating lymphocyte staining of samples from glioblastoma patients demonstrated elevated PD-1 expression by $CD4^+$ and $CD8^+$ T cells compared with normal donor patients, strongly suggestive of local, functional T cell defects (**Figure 1**). The proprietary PD-1/PD-L1 IHC staining by Merck has also definitively revealed clear expression of PD-1 on lymphocytic infiltrates within recurrent glioblastoma tissue (**Figure 2**). Thus, our studies document a discrete target for therapeutic intervention in patients with a deadly disease, with pre-clinical evidence of mechanism and therapeutic benefit. Advances in tumor immunotherapy may be of tremendous importance and relevance to primary brain tumors, particularly if these modulators of immunity have the capability of tracking and destroying infiltrating tumor cells.

Tissue based biomarker: Based on our pre-clinical findings in glioblastoma, as well as the findings of our colleagues at UCLA studying MK-3475 in melanoma patients, we believe that a novel tumor tissue-based assay will represent a valid pharmacodynamic biomarker for the bioactivity of this new agent in tumor tissue of glioblastoma patients for this proposed clinical trial. This new PCR-based assay will quantitatively assess the density of T lymphocytes within tumor tissue and the T cell receptor (TCR) clonality (3). Unpublished data from UCLA strongly suggests that TIL density and TCR clonality can be reliably quantified from tumor tissue and are correlated with clinical responses to MK-3475 in melanoma patients (**Fig. 3**). Such data also suggest that not only is the number of T lymphocytes important, but also the diversity for the response to MK-3475. These data are insightful as objective responses are known to occur with PD-1 inhibition in melanoma, however, it is unclear if any clinical or, for that matter, tissue biomarker responses might occur with PD-1 in the setting of recurrent/progressive glioblastoma. Therefore, more fundamental questions need to be addressed in investigations of PD-1 checkpoint inhibition in glioblastoma. Using an innovative clinical trial design, we will be able to test whether PD-1 checkpoint inhibition leads to increased TIL density in recurrent/progressive glioblastoma compared to tumor samples not exposed to PD-1 checkpoint inhibition.

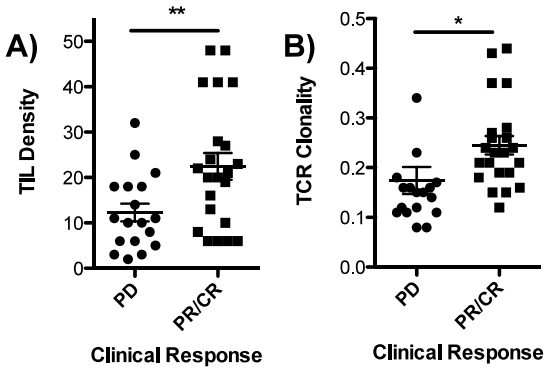

Figure 3: The estimated % T cell frequency (TIL Density, A) and TCR clonality (B) are significantly associated with clinical response to MK-3475 in melanoma patients. PD, progressive disease; PR/CR, partial response or complete response, as determined by RECIST criteria. (unpublished data supplied by Dr.'s Paul Tumeh and Antoni Ribas, UCLA Division of Hematology-Oncology).

2.3.2 Rationale for Dose Selection/Regimen/Modification

Available PK results in subjects with melanoma, NSCLC, and other solid tumor types support a lack of meaningful difference in PK exposures obtained at a given dose among tumor types. An open-label Phase I trial (Protocol 001) has been conducted to evaluate the safety and clinical activity of single agent pembrolizumab.[69] The dose escalation portion of this trial evaluated three

dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of pembrolizumab showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No maximum tolerated dose (MTD) has been identified.

In KEYNOTE-001, two randomized cohort evaluations of melanoma subjects receiving pembrolizumab at a dose of 2 mg/kg versus 10 mg/kg Q3W have been completed. The clinical efficacy and safety data demonstrate a lack of clinically important differences in efficacy response or safety profile at these doses. For example, in Cohort B2, advanced melanoma subjects who had received prior ipilimumab therapy were randomized to receive pembrolizumab at 2 mg/kg versus 10 mg/kg Q3W. The overall response rate (ORR) was 26% (21/81) in the 2mg/kg group and 26% (25/79) in the 10 mg/kg group (full analysis set (FAS)). The proportion of subjects with drug-related adverse events (AEs), grade 3-5 drug-related AEs, serious drug-related AEs, death or discontinuation due to an AE was comparable between groups or lower in the 10 mg/kg group. Population PK data analysis of pembrolizumab administered Q2W confirmed the expectation that intrinsic factors do not affect exposure to pembrolizumab to a clinically meaningful extent and also revealed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q3W dosing schedule. Taken together, these data also support the use of lower doses (with similar exposure to 2 mg/kg Q3W) in all solid tumor indications. 2 mg/kg Q3W is being evaluated in NSCLC in PN001, Cohort F30 and PN010, and 200 mg Q3W is being evaluated in head and neck cancer in PN012, which are expected to provide additional data supporting the dose selection.

Selection of 200 mg as the appropriate dose for a switch to fixed dosing is based on simulation results indicating that 200 mg will provide exposures that are reasonably consistent with those obtained with the 2 mg/kg dose and importantly will maintain individual patient exposures within the exposure range established in melanoma as associated with maximal clinical response. A population PK model, which characterized the influence of body weight and other patient covariates on exposure, has been developed using available data from 476 subjects from PN001. The distribution of exposures from the 200 mg fixed dose are predicted to considerably overlap those obtained with the 2 mg/kg dose, with some tendency for individual values to range slightly higher with the 200 mg fixed dose. The slight increase in PK variability predicted for the fixed dose relative to weight-based dosing is not expected to be clinically important given that the range of individual exposures is well contained within the range of exposures shown in the melanoma studies of 2 and 10 mg/kg to provide similar efficacy and safety. The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different tumor types and indication settings. A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and reduce potential for dosing errors. Additionally, a fixed dosing scheme will reduce complexity in the logistical chain at treatment facilities and reduce wastage.

2.3.3 Rationale for Endpoints

2.3.3.1 Efficacy Endpoints

Efficacy will be a secondary endpoint measured as percent PFS6.

Progression will be determined as per modified RANO criteria (iRANO)[104] as assessed by the investigator will be used for efficacy endpoints (Section 10). RANO will also be used by the local site to determine eligibility and make treatment decisions.

2.3.3.2 Safety Endpoints

An important primary objective of this study is to characterize the safety and tolerability of pembrolizumab when administered in the pre and post operative setting in surgically resectable recurrent glioblastoma. The safety analysis will be based on subjects who experience toxicities as defined by CTCAE 5.0 criteria. Safety will be assessed by quantifying the toxicities and grades experienced by subjects who have received pembrolizumab including serious adverse events (SAEs) and events of clinical interest (ECIs).

Safety will be assessed by reported adverse experiences using CTCAE, Version 5.0. The attribution to drug, time-of-onset, duration of the event, its resolution, and any concomitant medications administered will be recorded. AEs will be analyzed including but not limited to all AEs, SAEs, and fatal AEs.

2.4 Correlative Studies Background

Additional biomarker research to identify factors important for pembrolizumab therapy will also be pursued. Immune biomarker studies will include immunophenotyping of systemic immune cell subsets, TIL Density and TCR overlap, multi-plex IHC to assess: 1) the proportion of PD-L1 expression on GFAP+ tumor cells versus myeloid cells (CD68+ or CD163+) within the tumor microenvironment, and Gene expression Signatures and somatic mutations in an attempt to develop immune signatures as outlined in Section 8.0.

3. PARTICIPANT SELECTION

Male/female subjects of at least 18 years of age with recurrent glioblastoma who have not received prior bevacizumab, and are candidates for surgical tumor debulking will be enrolled in this trial.

Screening evaluations are detailed in Study Calendar (Section 9). All assessments are to occur within 14 days of registration except where otherwise noted. The participant must be thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the participant prior to enrollment.

Following registration, any additional laboratory assessments obtained prior to start of treatment will not be used to re-confirm eligibility. Please refer to Section 5.2 Dosing Delays/Dose

Modifications for toxicity management between registration and start of study treatment.

3.1 Eligibility Inclusion Criteria

In order to be eligible for participation in this trial, all subjects must meet the following criteria on screening examination:

3.1.1 Have histologically confirmed World Health Organization Grade IV malignant glioma (glioblastoma or gliosarcoma). Participants will be eligible if the original histology was low-grade glioma and a subsequent histological diagnosis of glioblastoma or variants is made.

3.1.2 Be willing and able to provide written informed consent/assent for the trial.

3.1.3 Be ≥ 18 years of age on day of signing informed consent.

3.1.4 Have a Karnofsky performance status (KPS) ≥ 70 (Appendix A).

3.1.5 Previous first line therapy with at least radiotherapy.

3.1.6 Be at first or second relapse. **Note**: Relapse is defined as progression following initial therapy (i.e., radiation ± chemotherapy). For participants who had prior therapy for a low-grade glioma, the surgical diagnosis of a high-grade glioma will be considered the first relapse.

3.1.7 Participants must have shown unequivocal evidence for tumor progression by MRI or CT scan.

3.1.8 Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 14 days of registration.

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥ 1.5 K/uL
Platelets	≥ 100 K/uL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X institutional upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	

Serum total bilirubin	≤ 1.5 X institutional ULN **OR**
	Direct bilirubin ≤ institutional ULN for subjects with total bilirubin levels > 1.5 institutional ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X institutional ULN **OR** ≤ 5 X institutional ULN for subjects with Gilberts syndrome
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X institutional ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X institutional ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

3.1.9 CT or MRI within 14 days prior of registration.
NOTE: Due to the fact that the screening MRI will not be used for response purposes, participants may be registered if screening CT or MRI is > 14 days of registration if prospective approval is received from Overall PI, Dr. Patrick Wen (for prospectively approved circumstances an eligibility exception will not need to be filed).

3.1.10 An interval of at least 4 weeks (to registration) between prior surgical resection or one week for stereotactic biopsy.

3.1.11 An interval of at least 12 weeks from the completion of radiation therapy to registration unless there is unequivocal histologic confirmation of tumor progression.

3.1.12 Participants must have recovered to grade 0 or 1 or pre-treatment baseline from clinically significant toxic effects of prior therapy (including but not limited to exceptions of alopecia, laboratory values listed per inclusion criteria, and lymphopenia which is common after therapy with temozolomide).

3.1.13 From registration, the following time periods must have elapsed: 5 half-lives from any investigational agent, 4 weeks from cytotoxic therapy (except 23 days for temozolomide and 6 weeks from nitrosoureas), 6 weeks from antibodies, or 4 weeks (or 5 half-lives, whichever is shorter) from other anti-tumor therapies (including vaccines). No wash-out period required from TTF.

3.1.14 Participants must have sufficient tissue from most recent surgery revealing glioblastoma or variants for submission following registration. The following amount of tissue is required:

- 1 formalin-fixed paraffin-embedded (FFPE) tumor tissue block

3.1.15 Patients with a prior or concurrent malignancy whose natural history or treatment does not have the potential to interfere with the safety or efficacy assessment of the trial are eligible. *NOTE: consultation with the Overall PI is highly recommended if enrollment of a patient with a prior or concurrent malignancy will be pursued.*

3.1.16 Patients must be undergoing surgery that is clinically indicated as determined by their care providers. Patients must be eligible for surgical resection with the expectation that the surgeon is able to resect at least 400mg of tumor with low risk of inducing neurological injury.

3.1.17 Female subjects of childbearing potential must have a negative urine or serum pregnancy within 72 hours prior to registration. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required. Women are considered post-menopausal and not of child bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g., age appropriate, history of vasomotor symptoms) or six months of spontaneous amenorrhea with serum FSH levels > 40 mIU/mL and estradiol < 20 pg/mL or have had surgical bilateral oophorectomy (with or without hysterectomy) at least six weeks ago. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of child bearing potential.

3.1.18 Women of child-bearing potential (WOCBP), defined as all women physiologically capable of becoming pregnant, must agree to use highly effective contraception during study treatment and for 120 days after study discontinuation. Highly effective contraception is defined as either:

i. True Abstinence: When this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

ii. Sterilization: Surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks ago. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment (as described in item 3.1.16 above).

iii. Male Partner Sterilization (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate). For female subjects on the study, the vasectomized male partner must be the sole partner for that participant.

iv. Use of a combination of any two of the following:

a. Placement of an intrauterine device (IUD) or intrauterine system (IUS)

b. Barrier methods of contraception: Condom or Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/vaginal suppository

c. Appropriate hormonal contraceptives (including any registered and marketed contraceptive agent that contains an estrogen and/or a progestational agent – including oral, subcutaneous, intrauterine, or intramuscular agents)

3.1.19 Male subjects must agree to use adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of therapy.

3.2 Eligibility Exclusion Criteria

The subject must be excluded from participating in the trial if the subject:

3.2.1 Current or planned participation in a study of an investigational agent or using an investigational device.

3.2.2 Has a diagnosis of immunodeficiency.

3.2.3 Has tumor primarily localized to the brainstem or spinal cord.

3.2.4 Has presence of diffuse leptomeningeal disease or extracranial disease.

3.2.5 Has received systemic immunosuppressive treatments, aside from systemic corticosteroids (such as methotrexate, chloroquine, azathioprine, etc) within six months of registration.

3.2.6 Has received anti-angiogenic or anti-VEGF targeted agents (e.g. bevacizumab, cedirinab, aflibercept, vandetanib, XL-184, sunitinib, etc).

3.2.7 Requires treatment with high dose systemic corticosteroids defined as dexamethasone > 4 mg/day or bioequivalent for at least 3 consecutive days within 2 weeks of registration.

3.2.8 Has received prior interstitial brachytherapy, implanted chemotherapy, stereotactic radiosurgery or therapeutics delivered by local injection or convection enhanced delivery.

3.2.9 Has history of known coagulopathy that increases risk of bleeding or a history of clinically significant hemorrhage within 12 months of registration.

3.2.10 Has a known history of active TB (Bacillus Tuberculosis).

3.2.11 Has gastrointestinal bleeding or any other hemorrhage/bleeding event CTCAE Grade > 3 within 6 months of registration.

3.2.12 Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

3.2.13 Has known history of, or any evidence of active non-infectious pneumonitis.

3.2.14 Has an active infection requiring systemic therapy.

3.2.15 Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator. Examples include but are not limited to symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.16 Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

3.2.17 Has received prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways).

3.2.18 Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).

3.2.19 Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).

3.2.20 Has received a live vaccine within 30 days prior to registration.

3.2.21 Has a known hypersensitivity to any of the study therapy products.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

All sites should email the Study Coordinator email to NeuroOnc_Coor@dfci.harvard.edu to verify slot availability.

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trial Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol-specific therapy or intervention. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the ODQ protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy and/or intervention. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If

a participant does not receive protocol therapy following registration, the participant's participant must be taken off-study in the CTMS (OnCore) with an appropriate date and reason entered.

4.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

See Appendix B Section 3.7 (DF/HCC Multi-Center Data and Safety Monitoring Plan) for complete registration procedures.

4.4 Registration Process for Other Investigative Sites

See Appendix B Section 3.7 (DF/HCC Multi-Center Data and Safety Monitoring Plan) for complete registration procedures.

5. TREATMENT PLAN

This is a pilot surgical trial to evaluate early immunologic pharmacodynamic parameters for the PD-1 checkpoint inhibitor, pembrolizumab (MK-3475), in patients with surgically accessible recurrent/progressive glioblastoma. The principle goal of this study is to understand, in surgically resectable recurrent/progressive GBM, if neoadjuvant pembrolizumab elicits a systemic immunologic response, a tumoral immunologic response and toxicity and if that response can be correlated with other non-invasive measures of pembrolizumab bioactivity (tumor, blood, imaging, and clinical outcomes). The study includes 2 stages, in the first stage (**Stage 1**), for optimal understanding of the effect of the drug on tumor tissue patients will be randomized in to Group A or Group B. In the second stage (**Stage 2**), additional patients will be enrolled to an expansion arm of Group A only to specifically study the effect of the drug on cell cycle/cancer proliferation related genetic signature.

Stage 1:

Groups A and B: Patients will be randomly assigned to either group A or B with a 1:1 randomization ratio prior to surgery. Patients in all groups will receive the study drug pembrolizumab after tumor resection. Only Group A will receive a single infusion of pembrolizumab of 200mg 14± 5 days prior to surgery (when scheduling, please keep in mind that the two pre-surgical plasma samples should be drawn greater than 10 days apart - see Table 6 for full details). Group B will not receive study drug prior to surgery. **This randomization scheme should not negatively impact study accrual and will provide an invaluable control cohort for PD evaluations.**

Group A: Neoadjuvant/adjuvant evaluation: Fifteen eligible patients will receive a single infusion of pembrolizumab of 200mg 14± 5 days prior to scheduled surgical resection for

recurrent/progressive glioblastoma (when scheduling, please keep in mind that the two pre-surgical plasma samples should be drawn greater than 10 days apart - see Table 6 for full details). Tumor samples will be obtained at time of surgery. The tissue from this surgery (fresh, frozen and FFPE) and the archived tissue from the archived tissue from the most recent surgery prior to registration revealing glioblastoma (FFPE) will be processed so as to best achieve the primary, secondary and exploratory PD objectives. After recovery from surgery (once participant has recovered from surgery, but no more than 35 days following surgery), patients will resume Q 3 week pembrolizumab at 200mg IV until tumor progression or adverse event requiring discontinuation of study drug. Blood samples will be obtained as pharmacodynamic markers throughout the study. Dose holds and symptomatic management will occur based upon preset adverse event determination. DLTs will not be determined. The toxicity evaluation period will begin with registration and extend to 30 days after last treatment day. Patients will be followed for MRI changes, clinical exam and steroid doses from the registration period and extend to the second progression. After second progression, patients will be followed every 3 months for vital status until death.

Group B: adjuvant evaluation: Fifteen eligible patients will not receive study drug prior to scheduled surgical resection for recurrent/progressive glioblastoma. The two required pre-surgical plasma samples should be drawn greater than 10 days apart - see Table 6 for full details). Tumor samples will be obtained at time of surgery. The tissue from this surgery (fresh, frozen and FFPE) and the archived tissue from the most recent surgery prior to registration revealing glioblastoma (FFPE) will be processed so as to best achieve the primary, secondary and exploratory PD objectives. After recovery from surgery (once participant has recovered from surgery, but no more than 35 days following surgery), patients will begin Q 3 week pembrolizumab at 200mg IV until tumor progression or adverse event requiring discontinuation of study drug. Blood samples will be obtained as pharmacodynamic markers throughout the study. Dose decreases and symptomatic management will occur based upon preset adverse event determination. DLTs will not be determined. The toxicity evaluation period will begin with registration and extend to 30 days after last treatment day. Patients will be followed for MRI changes, clinical exam and steroid doses from the registration period and extend to the second progression. After second progression, patients will be followed every 3 months for vital status until death.

Stage 2:

Group A expansion only: For this stage of the study, 25 additional patients will be enrolled to Group A only with a goal of 20 evaluable patients.

Expansion of Group A: Neoadjuvant/adjuvant evaluation: An additional 20-25 patients will receive a single infusion of pembrolizumab of 200mg 14± 5 days prior to scheduled surgical resection for recurrent/progressive glioblastoma (when scheduling, please keep in mind that the two pre-surgical plasma samples should be drawn greater than 10 days apart - see Table 6 for full details). Tumor samples will be obtained at time of surgery. The tissue from this surgery (fresh, frozen and FFPE) and the archived tissue from the archived tissue from the most recent surgery prior to registration revealing glioblastoma (FFPE) will be processed so as to best achieve the primary, secondary and exploratory PD objectives. After recovery from surgery (once participant has recovered from surgery, but no more than 35 days following surgery), patients will resume Q 3 week pembrolizumab at 200mg IV until tumor progression or adverse

event requiring discontinuation of study drug. Blood samples will be obtained as pharmacodynamic markers throughout the study. Dose holds and symptomatic management will occur based upon preset adverse event determination. DLTs will not be determined. The toxicity evaluation period will begin with registration and extend to 30 days after last treatment day. Patients will be followed for MRI changes, clinical exam and steroid doses from the registration period and extend to the second progression. After second progression, patients will be followed every 3 months for vital status until death.

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of trial treatments in accordance with the protocol and any applicable laws and regulations.

5.1 Trial Treatments

The treatment to be used in this trial is outlined below. Each treatment cycle will be 6 weeks (42 days).

Table 2 Trial Treatment

Drug	Dose/ Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Days 1 and 22 of each 6-week (42 day) cycle	Experimental

5.2 Dose Selection/Modification

Dose Selection

The rationale for selection of doses to be used in this trial is provided in Section 2.3.2 – Background and Rationale.

Details on preparation and administration of pembrolizumab (MK-3475) are provided in the Pharmacy Manual.

Dose Modification

No dose reductions (in dose or frequency) are permitted. Only dose holds are permitted per protocol. Treatment may be held for up to 12 weeks (84 days) from last dose.

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities (considered at least possibly related to study treatment) and severe or life-threatening AEs as per Table 3 below. If a participant experiences a toxicity unlikely or unrelated to treatment

with pembrolizumab, but may still warrant a hold or reduction of study drug for safety, discussion and writing approval by the Overall Principal Investigator and Study Sponsor, Patrick Wen, MD, is recommended but not required.

See Section 5.7 for supportive care guidelines, including use of corticosteroids.

Table 3 Dose Modification Guidelines for Drug-Related Adverse Events

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1.	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose.
	3-4	Permanently discontinue (see exception below)[1]	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure.	Resume pembrolizumab when patients are clinically and metabolically stable within 12 weeks of last dose.
Hypophysitis	2-4	Toxicity resolves to Grade 0-1. Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism	2-4	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted.
Immune system disorders - Other: Guillain-Barre syndrome - Any Grade		Permanently discontinue	Permanently discontinue
Infusion Reaction	2[2]	Toxicity resolves to Grade 0-1	Permanently discontinue if toxicity develops despite adequate premedication
	3-4	Permanently discontinue	Permanently discontinue
Myocarditis	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose
	4	Permanently discontinue	Permanently discontinue
Myositis	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose
	4	Permanently discontinue	Permanently discontinue
Neuropathy (peripheral motor or sensory)	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose
	4	Permanently discontinue	Permanently discontinue
Pancreatitis	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose
	4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue
Stevens-Johnson Syndrome	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[3]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.
[1] For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued.
[2] If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose; Refer to

Table – Infusion Treatment Guidelines for further management details.
[3]Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

5.3 Timing of Dose Administration

For Group A pre-surgery, participants will receive a single infusion of pembrolizumab 200mg 14 +/- 5 days prior to scheduled surgical resection (when scheduling, please keep in mind that the two pre-surgical plasma samples should be drawn greater than 10 days apart - see Table 6 for full details) .

For Group A and B after recovery from surgery (once participant has recovered from surgery, but no more than 35 days following surgery), trial treatment should be administered on Days 1 and 22 of each 6 week cycle after all procedures/assessments have been completed and reviewed as detailed on the Trial Flow Chart (Section 9). Trial treatment may be administered up to 3 days before or after the scheduled day of each cycle due to administrative reasons.

All trial treatments will be administered on an outpatient basis.

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible.

However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

5.4 Trial Blinding/Masking

This is an open-label trial; therefore, the Sponsor, investigators and subject will know the treatment administered.

5.5 Stratification

No stratification based on age, gender or other characteristic will be used in this trial.

5.6 Patient Evaluability and Replacement

See protocol section 12.2.5 Analysis Population.
If a participant is enrolled to trial and local pathology from the on-study surgery is unable to definitively confirm recurrent tumor, then the participant will be considered non-evaluable for primary and secondary endpoints, but may continue to receive study treatment per protocol. In these instances, please contact the DFCI Coordinating Center as soon as possible to obtain Sponsor-Investigator and manufacturer approval.

5.7 General Concomitant Medication and Supportive Care Guidelines

Medications or vaccinations specifically prohibited in the exclusion criteria (Section 3.2) are not allowed during the ongoing trial except as outlined below. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The treating investigator should discuss any questions regarding this with the sponsor and the overall study PI or his designee. The final decision on any supportive therapy or vaccination rests with the treating. However, the decision to continue the subject on trial therapy or vaccination schedule requires the mutual agreement of the Investigator, Sponsor, overall study PI or his designee, and the subject.

5.7.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF, when information is available.

All concomitant medications received from date of consent up to 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs.

5.7.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Anti-cancer systemic chemotherapy or biological therapy, with the exception of bevacizumab for the purpose of symptomatic management or to control cerebral edema (see section 5.8)
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab

- Radiation therapy
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology or to control cerebral edema. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor and Overall PI, Dr. Patrick Wen.
-

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.8 Rescue Medications & Supportive Care

Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 5.2 for dose modification.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

- **Pneumonitis**:
    - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
        - Recommended corticosteroid regimen:
            - 1-2mg/kg/day prednisone or equivalent

- When symptoms resolve to grade 1 or less, initiate steroid taper for no less than 4 weeks

- For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
  - Recommended corticosteroid regimen:
    - Methylprednisolone 125mg IV daily
    - When symptoms grade 1 or less initiate steroid taper for no less than 4 weeks
      - Prednisone 1-2 mg/kg/day or dexamethasone 4mg every 4 hours
    - If IV steroids do not reduce initial symptoms within 48-72 hours, treat with additional anti-inflammatory measures
- Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

- **Diarrhea/Colitis**:
  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

  - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
  - For **Grade 2 diarrhea/colitis**, administer oral corticosteroids.
    - Recommended corticosteroid regimen:
      - Prednisone 1-2mg/kg/day or equivalent
      - When symptoms grade 1 or less, initiate steroid taper for no less than 4 weeks
  - For **Grade 3 or 4 diarrhea/colitis**, treat with intravenous steroids followed by high dose oral steroids.
    - Recommended corticosteroid regimen:
      - Methylprednisolone 125mg IV daily
      - When symptoms grade 1 or less initiate steroid taper for no less than 4 weeks and in 6-8 weeks in patients with diffuse or severe ulceration and/or bleeding
        - Prednisone 1-2 mg/kg/day or dexamethasone 4mg every 4 hours
      - If IV steroids do not reduce initial symptoms within 48-72 hours treat with additional anti-inflammatory measures
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**
    - For **T1DM** or **Grade 3-4** Hyperglycemia
        - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
        - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis**:
    - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
    - For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hyperthyroidism or Hypothyroidism**:
  Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.
    - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
        - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
        - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
    - **Grade 3-4** hyperthyroidism
        - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hepatic**:
    - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
        - Treat with IV or oral corticosteroids
    - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
    - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Renal Failure or Nephritis**:
    - For **Grade 2** events, treat with corticosteroids.

- For **Grade 3-4** events, treat with systemic corticosteroids.
- When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

Table 4 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 4 Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

5.8.1 Cerebral Edema

Due to the immunologic nature of pembrolizumab administration, cerebral edema could theoretically result as a consequence of pembrolizumab administration due to immune infiltration of the brain. Symptoms related to cerebral edema may include headache or neurologic deficits that are either new or worsened. Patients with any signs or symptoms of cerebral edema should be treated as clinically appropriate including initiation or increased systemic corticosteroid dosing, initiation of bevacizumab administered per local institutional practice, treatment with an osmotic diuretic or surgical decompression. An investigator may initiate bevacizumab per local institutional practice if significant mass effect is seen on an MRI in anticipation of subsequent clinical decline. Subsequent pembrolizumab dosing should be immediately interrupted if significant clinical symptoms attributable to cerebral edema develop. Treatment with additional pembrolizumab doses may only be re-initiated if clinically significant symptoms attributable to cerebral edema have resolved to grade ≤ 1 or pre-treatment baseline. Patients who develop CTCAE 5.0 grade 4 cerebral edema attributable to pembrolizumab administration should not receive further pembrolizumab doses and should discontinue study therapy.

NOTE: every effort should be made to not exceed a corticosteroid dose of > 4 mg / day between date of registration and on-study surgery. If a corticosteroid of > 4 mg / day is necessary a participant following registration and prior to on-study surgery, the treating investigator should reach out to the Overall PI to confirm the participant's evaluability for tumor tissue studies.

5.8.2 Diet/Activity/Other Considerations

5.8.2.1 Diet

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.

5.8.2.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or 3) not heterosexually active for the duration of the study. The two birth control methods can be either two barrier methods or a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy.

The following are considered adequate barrier methods of contraception: diaphragm, condom (by the partner), copper intrauterine device, sponge, or spermicide. Appropriate hormonal contraceptives will include any registered and marketed contraceptive agent that contains an

estrogen and/or a progestational agent (including oral, subcutaneous, intrauterine, or intramuscular agents).

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study they must adhere to the contraception requirement (described above) for the duration of the study and during the follow-up period defined in section 6.2 Reporting of Pregnancy and Lactation to the Sponsor and to Merck. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.8.2.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to Merck without delay and within 1 working day if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to Merck and followed as described above and in Section 6.2.

5.8.2.4 Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

5.9 Duration of Therapy and Criteria for Removal from Study Treatment

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. Treatment may continue until one of the following criteria applies:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Confirmed radiographic disease progression
  Note: For unconfirmed radiographic disease progression, please see Section 10.5
  Note: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved, please see Section 10.5.
- Unacceptable adverse experiences
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test

- Noncompliance with trial treatment or procedure requirements
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator
- Administrative reasons

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

When a participant is removed from protocol therapy and/or is off of the study, the relevant Off-Treatment/Off-Study information will be updated in OnCore.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Patrick Wen, M.D.

5.10 End of Treatment Evaluation and Follow-Up

The End of Treatment and Follow-up visit procedures are listed in Section 9 (Study Calendar). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring and 90 days for serious adverse event reporting.

Participants removed from protocol therapy for unacceptable adverse events will be following until resolution or stabilization of the adverse event.

Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up.

5.11 Long-Term Follow-Up and Study Completion

After documented disease progression each subject will be followed by telephone or medical record review for overall survival until they meet criteria for removal from study as detailed below.

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF).

When a participant is removed from protocol therapy and/or is off of the study, the relevant Off-Treatment/Off-Study information will be updated in OnCore.

5.12 Subject Replacement Strategy

Additional subjects may be enrolled in a given Group to ensure that the required number of evaluable subjects in each cohort is achieved. A subject that discontinues the trial for progressive disease or a drug-related AE will not be replaced and will be counted in the evaluable population of subjects for the respective cohort.

5.13 Beginning and End of the Trial

The study begins when the first subject signs the informed consent (either pre-screening consent or main study consent). The end of the study may be designated as the time point when all subjects have discontinued the study or are a minimum of 6 months post initial study medication administration. If, by the end of the study, there remains at least 1 subject still on study treatment for at least 6 months, the subject(s) may enter additional treatment cycles.

The subject is considered on study until such time that he/she meets any of the discontinuation criteria and notification is given to the Sponsor.

6. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Merck's product, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to onset of menses or menopause occurring at a physiologically appropriate time.

Merck product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by Merck for human use.

Adverse events may occur during the course of the use of Merck product in clinical trials or within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Adverse events may also occur in screened subjects during any pre-allocation baseline period as a

result of a protocol-specified intervention, including washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Progression of the cancer under study is not considered an adverse event.

All adverse events will be recorded from registration through 30 days following cessation of treatment and at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 6.3.

Adverse events will not be collected for subjects during the pre-screening period (for determination of archival tissue status) as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

6.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 1 working day hours to the DFCI Coordinating Center and Overall PI (Dr. Patrick Wen) and within 2 working days hours (see Appendix C DFCI Reportable AE Coversheet) to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215 993-1220). The DFCI Neuro-Oncology Coordinating Center central SAE email should be copied on the submission (NeuroOnc_SAE@dfci.harvard.edu).

6.2 Reporting of Pregnancy and Lactation to the Sponsor and to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment

allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of Sponsor's product, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 1 working day to the DFCI Coordinating Center and Overall PI (Dr. Patrick Wen – see Appendix C DFCI Reportable AE Coversheet) and within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215 993-1220). The DFCI Neuro Oncology Coordinating Center central SAE email should be copied on the submission (NeuroOnc_SAE@dfci.harvard.edu).

6.3 Immediate Reporting of Adverse Events to the Sponsor and to Merck

6.3.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of Merck's product that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is another important medical event

Refer to Table 5 for additional details regarding each of the above criteria.

Progression of the cancer under study is not considered an adverse event unless it results in hospitalization or death. See section 6.3.1.5 Protocol Specific Expedited Adverse Event Reporting Exclusions for other events not requiring expedited reporting.

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported within 1 working day to the DFCI Coordinating Center (see Appendix C DFCI Reportable AE Coversheet) and Overall PI and within 2 working days to Merck Global Safety.

Non-serious Events of Clinical Interest will be forwarded to Merck Global Safety and will be handled in the same manner as SAEs.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to the DFCI Coordinating Center, Overall PI and to Merck.

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-993-1220

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220) at the time of submission to FDA.

All subjects with serious adverse events must be followed up for outcome.

Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 90 days of the last dose of treatment (or the initiation of new anti-cancer therapy, whichever is earlier) on the local institutional SAE form.

For multi-institution studies where a DF/HCC investigator is serving as the Overall Principal Investigator, each participating institution **must** abide by the reporting requirements set by the DF/HCC. This applies to any medical event equivalent to an unexpected grade 2 or 3 with a possible, probable or definite attribution, unexpected grade 4 toxicities, and grade 5 (death) regardless of study phase or attribution.

6.3.1.1 DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC and DF/PCC will report SAEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

Other investigative sites will report SAEs to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional SAE form should be forwarded to the Overall PI within the timeframes detailed in the table below.

Attribution	DF/HCC Reportable AEs			
	Gr. 2 & 3 AE Expected	Gr. 2 & 3 AE Unexpected	Gr. 4 AE Unexpected	Gr. 5 AE Expected or Unexpected
Unrelated Unlikely	Not required	Not required	5 calendar days	1 working day
Possible Probable Definite	Not required	5 calendar days	5 calendar days	1 working day
* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 90 days of the last intervention (or the initiation of new anti-cancer therapy, whichever is earlier), the AE should be reported within 1 working day of learning of the event.				

The Overall PI will submit SAE reports from outside institutions to the DFCI OHRS according to DFCI IRB policies and procedures in reporting adverse events.

6.3.1.2 Expedited Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

6.3.1.3 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

6.3.1.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.**

6.3.1.5 Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting to the Overall PI, the DFCI IRB or Merck. However, they still must be reported through the routine reporting mechanism (i.e. case report form).

- Lymphopenia (grades 2-4)
- A visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- Emergency outpatient treatment for an event not fulfilling the serious criteria outlined above in section 6.3.1 and not resulting in inpatient admission
- Elective surgery, planned prior to signing consent
- Routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy)

- Hospitalization for routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- Hospitalization for seizure, if felt related to patient's underlying disease
- Hospitalization for elective or pre-planned treatment for a pre-existing condition that did not worsen
- Hospitalization for respite care not associated with an adverse event attributed to the study drug
- Hospitalization for surgical intervention in order to delineate pseudoprogression due to inflammation associated with PD-1 blockade from true tumor progression as mentioned in section 10.5.

6.3.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must reported within 1 working day to the DFCI Coordinating Center and Overall PI and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 6.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is more than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

ECIs that occur in any subject from the date of first dose through 90 days following cessation of treatment, or the initiation of a new anticancer therapy, whichever is earlier, whether or not related to the Merck's product, must be reported within 1 working day to the DFCI Coordinating Center and Overall PI and to Merck Global Safety within 2 working days.

6.4 Evaluating Adverse Events

An investigator will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 5.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness as defined in Section 6.3.1 and Table 5. In addition, attribution of AEs must be classified based on the following definitions:

Definite – The AE *is clearly related* to the study treatment.
Probable – The AE *is likely related* to the study treatment.
Possible – The AE *may be related* to the study treatment.
Unlikely – The AE *is doubtfully related* to the study treatment.
Unrelated – The AE *is clearly NOT related* to the study treatment.

In order for an event to be considered expected (known correlation to study drug) for the purposes of adverse event reporting, the event must be included in the section below. This list of adverse events categorized by CTCAE v. 5.0 is derived from the current pembrolizumab Investigator Drug Brochure. If a treating investigator would like to consider an adverse event expected that is not listed below, discussion with the DFCI Coordinating Center, is required.

- **BLOOD AND LYMPHATIC SYSTEM DISORDERS** *: anemia
- **CARDIAC DISORDERS**: heart failure; myocarditis
- **ENDOCRINE DISORDERS**: adrenal insufficiency; hyperthyroidism; hypothyroidism;
    - Endocrine disorders – other, specify: hypophysitis; thyroiditis; type 1 diabetes mellitus
- **EYE DISORDERS** *: uveitis;
    - Eye disorders – other, specify: iritis
- **GASTROINTESTINAL DISORDERS** *: abdominal pain; colitis; constipation; diarrhea; nausea; pancreatitis; vomiting
- **GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS**: edema limbs; fatigue; fever; infusion related reaction
- **HEPATOBILIARY DISORDERS** *:
    - Hepatobiliary disorders– other, specify: hepatitis, sclerosing cholangitis
- **IMMUNE SYSTEM DISORDERS**: allergic reaction
- **INFECTIONS AND INFESTATIONS**: skin infection; urinary tract infection; upper respiratory infection;
    - Infections and infestations – other, specify: pneumonia
- **INJURY, POISONING AND PROCEDURAL COMPLICATIONS ***
- **INVESTIGATIONS:** activated partial thromboplastin time prolonged; alanine aminotransferase increased; alkaline phosphatase increased; aspartate aminotransferase increased; blood bilirubin increased; creatinine increased; GGT increased; INR increased; neutrophil count decreased; platelet count decreased; weight loss
- **METABOLISM AND NUTRITION DISORDERS**: anorexia; hypoalbuminemia; hyponatremia
- **MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS**: myalgia; arthralgia; arthritis; myositis; Rhabdomyolysis
    - Musculoskeletal and connective tissue disorders – Other, specify: myopathy; rejection of solid organ or tissue transplants (corneal, kidney, or liver)
- **NERVOUS SYSTEM DISORDERS** *: dizziness; dysgeusia; edema cerebral; headache; peripheral motor neuropathy; peripheral sensory neuropathy; seizures;

  - Nervous system disorders – other, specify: Guillain-Barre syndrome; myasthenia gravis (MG)
- **PSYCHIATRIC DISORDERS:** confusion
- **RENAL AND URINARY DISORDERS** *: hematuria;
  - Renal and urinary disorders – other, specify: nephritis
- **REPRODUCTIVE SYSTEM AND BREAST DISORDERS** *
- **RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS** *: cough; dyspnea; pneumonitis
- **SKIN AND SUBCUTANEOUS TISSUE DISORDERS**: exfoliative dermatitis
  - All CTCAE rashes are considered expected: bullous dermatitis; erythema multiforme; erythroderma; palmar-plantar erythrodysesthesia syndrome; pruritus; rash acneiform; rash maculo-papular; skin hyperpigmentation; skin hypopigmentation; skin induration; skin ulceration; Stevens-Johnson syndrome; toxic epidermal necrolysis (TEN) ;
  - Skin and subcutaneous tissue disorders – other, specify: pemphigus; vitiligo
- **VASCULAR**: vasculitis

* In addition, the following adverse event is considered expected within all applicable SOCs:
- bleeding/hemorrhage

Table 5 Evaluating Adverse Events

Appropriately delegated study team members will evaluate all adverse events as to:

V5.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer;** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable to the Sponsor within 24 hours and to Merck within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours to the Sponsor and to Merck within 2 working days..	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause the Merck product to be discontinued?	
Relationship to Merck Product	Did Merck product cause the adverse event? The determination of the likelihood that Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to Merck product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Merck product; or (3) the trial is a single-dose drug trial); or (4) Merck product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to Merck product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Merck product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY MERCK PRODUCT, OR IF REEXPOSURE TO MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following	**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Merck product relationship).**	
Yes, there is a reasonable possibility of Merck product relationship.	There is evidence of exposure to Merck product. The temporal sequence of the AE onset relative to the administration of Merck product is reasonable. The AE is more likely explained by Merck product than by another cause.	
No, there is not a reasonable possibility Merck product relationship	Subject did not receive Merck product OR temporal sequence of the AE onset relative to administration of Merck product is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)	

6.5 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

7. AGENT INFORMATION

7.1 Investigational Product: Pembrolizumab

Pembrolizumab is a potent humanized IgG4 mAb with high specificity of binding to the PD-1receptor, thus inhibiting its interaction with PD-L1 and PD-L2. Based on preclinical in vitro data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1. Pembrolizumab has an acceptable preclinical safety profile and is being advanced for clinical development as an IV immunotherapy for advanced malignancies.

Additional detailed information on pembrolizumab is available in the Investigator's Brochure.

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

7.1.1 Description

Pembrolizumab is a humanized anti-PD-1 mAb of the IgG4/kappa isotype with a stabilizing S228P sequence alteration in the fragment crystallizable (Fc) region. Pembrolizumab binds to human PD-1 and blocks the interaction between PD-1 and its ligands. The theoretical molecular weight of the polypeptide is 146,288 Da and its theoretical pI is 7.5. Pembrolizumab exhibits linear pharmacokinetics at dose levels of clinical relevance (1-10 mg/kg). It exhibits low clearance and limited volume of distribution that is typical for therapeutic antibodies. Mean estimated t1/2 values are 14.1-21.6 days. Additional information on pembrolizumab nomenclature is detailed in the following table:

Code Name	Pembrolizumab (anti-PD-1)
Other Code Name	SCH 900475 (anti-PD-1) MK-3475
Chemical Name	Humanized X PD-1 mAb (H409A11) IgG4
CAS Number	1374853-91-4
CAS Name	Anti-(human protein PDCD1 (programmed cell death 1)) immunoglobulin G4 (human-Mus musculus monoclonal heavy chain) disulfide with human-Mus musculus monoclonal light chain, dimer
Generic Name	Pembrolizumab
Commercial Name	Keytruda®

7.1.2 Form

Pembrolizumab is supplied as a clear to opalescent solution that is essentially free of extraneous particles and may contain proteinaceous particulates. One dosage form of pembrolizumab will be provided by Merck in Type I glass vials intended for single use only as summarized in the following table:

Product Name & Potency	Dosage Form
Pembrolizumab 100 mg/ 4mL	Solution for Infusion

Pembrolizumab solution for infusion is a sterile, non-pyrogenic, aqueous, preservative-free solution. Pembrolizumab solution for infusion contains an excess fill of 6.25 mg (equivalent to 0.25 mL solution) to ensure the recovery of label claim of 100 mg pembrolizumab per vial (equivalent to 4.0 mL of solution).

7.1.3 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements. Vials will be provided in an open label fashion for subject dosing.

7.1.4 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

7.1.5 Storage, Handling and Preparation Requirements

As specified in the Pharmacy Manual for pembrolizumab as provided by the Sponsor.

7.1.6 Administration

Pembrolizumab will be administered as a 30-minute IV infusion using an infusion pump (treatment cycle intervals may be increased due to toxicity as described in Section 6.2). Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min). Attach the infusion line to the pump and prime the line, either with normal saline (at least 25 mL) or with infusion solution as per local SOP, before starting the infusion. Maximum infusion rate should not exceed 6.7 ml/min through a peripheral or indwelling catheter. Use 30 mL normal saline to flush the infusion line at the end of the infusion if institutional guidelines allow.

Unused infusion solution should not be used for another infusion of the same participant or different participant.

DO NOT administer the product as an intravenous push or bolus.

DO NOT combine, dilute or administer it as an infusion with other medicinal products.

A central line is not required for pembrolizumab administration, but may be used if available.

The following infusion set materials are compatible with pembrolizumab

- PVC infusion set that is plasticized using Di-2-ethylhexyl Terephthalate DEHP
- PVC and tri-(2-ethylhexyl) trimellitate (TOTM) infusion set
- Polyehtylene lined PVC infusion set
- Polyrethane
- Plybutadiene

A sterile, non-pyrogenic, low-protein binding 0.2 to 5 µm in-line filter made of polyethersulfone (PES) or polysulfone must be used during administration to remove any adventitious particles. If the infusion set does not contain a 0.2 to 5 µm in-line filter, it is recommended to use a 0.2 to 5 µm add-on filter which may contain an extension line (the materials of the extension line and filter should be as mentioned above).

7.1.7 Ordering

Investigative sites will order and acquire Pembrolizumab directly from Merck per Appendix B Section 4.0 (Data and Safety Monitoring Plan).

7.1.8 Accountability

The investigator is responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial.

7.1.9 Destruction and Return

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

8. BIOMARKER STUDIES

The precise mechanism by which Pembrolizumab exerts anti-tumor activity is not clear but it is likely related to modulation of the immune system to generate anti-tumor immune responses that are capable of eliminating existing tumors and generating immune memory responses to prevent future relapse. The effect of PD-1 blockade on effector T cells and regulatory T cells is a critical component of anti-tumor activity. The current study will explore baseline levels as well as changes following pembrolizumab administration for association with outcome. In addition, archival tumor expression of PD-L1 will also be evaluated. Peripheral blood will be collected prior to initiation of study therapy, prior to surgery and periodically during the study. Archival tumor will also be collected on all patients. Since this is a surgery study, all tumor samples obtained during the study will be use to evaluate effect of therapy. In addition, if a biopsy or surgical resection is performed at the time of potential progression, a tumor sample (block or slides) should also be submitted if sufficient tumor material is available.

Evaluation of correlative immunologic biomarkers will be performed at the Brain Tumor Immunology Research Laboratory, UCLA Jonsson Comprehensive Cancer Center. Instructions for sample processing and shipping are provided in this section.

Please note that submitting institution is responsible for the costs of shipping and handling.

8.1 Immunophenotyping

The absolute lymphocyte count and proportion of specific lymphocyte subsets will be quantified at each time point. Using fluorescence activated cell sorting (FACS) analysis, lymphocyte/leukocyte subsets, activation markers, and negative costimulatory molecules will be evaluated before and after administration of pembrolizumab. The CD4/CD8 ratio, Treg populations (CD3+CD4+CD25+CD127low), activation (CD3+CD8+CD25/69), MDSC (CD33+HLA-DrlowCD11b+PD-L1+), negative costimulatory markers (CD3+CD4/8+PD-1+, CD3+CD4/8+CTLA-4+) will be determined at each time point. FACS analysis will be performed on PBMC obtained from Ficoll density gradient separation of whole blood. Blood draws for this testing will be done pre treatment, pre-surgery, and with every MRI scan done for tumor status. Guidance on peripheral blood collection, processing and shipping is provided in Table 6 below.

8.2 TIL Density and TCR Overlap

In this study we plan to evaluate whether next generation sequencing of the T cell receptor (TCR) repertoire within GBM and blood can identify shared TCRs, which can then effectively track anti-tumor immune responses induced by PD-1 mAb blockade. Genomic DNA will be isolated from fresh-frozen tumor (protocol surgery) and peripheral blood (immune monitoring timepoints) and subjected to next generation sequencing through the TCRVβ region to quantify TIL density and assess the overlap between tumor and peripheral blood. The TIL density and TCR overlap will then be correlated with clinical variables to identify potential biomarkers with prognostic and predictive value for outcomes (ORR, PFS, OS and toxicity). The TIL density and TCR overlap will be performed at Adaptive Biotechnologies (Seattle, WA). Investigators at the UCLA Brain Tumor Immunology Research Laboratory will perform analysis. Guidance on peripheral blood

collection, processing and shipping is provided in Table 6 below.

8.3 IHC Measurements

A minimum of 1 formalin-fixed paraffin-embedded (FFPE) tumor tissue block from pre-study surgery confirming GBM are to be submitted within 60 days of registration. Additionally, a minimum 1 formalin-fixed paraffin-embedded (FFPE) tumor tissue block (preferred) or a minimum of 10 FFPE unstained sections from the protocol surgery are to be submitted per Table 6 below. Archival tumor samples from the protocol surgery should be shipped to the UCLA Brain Tumor Research Lab. Multi-plex IHC stained will be performed to assess: 1) the proportion of PD-L1 expression on GFAP+ tumor cells versus myeloid cells (CD68+ or CD163+) within the tumor microenvironment and subsequently batch shipped to a central lab determined by Merck for determination of PD-L1 expression at the end of the study.

NOTE: archival tumor tissue from pre-study surgery and protocol surgery submitted to the DFCI Coordinating Center outside of protocol mentioned timelines will not be logged as a violation. Sites will be tracked for compliance, but IRB reporting of out of window submission will not be required.

8.4 Gene expression signatures and somatic mutations

Tumor samples from the protocol surgery should be immersed in Allprotect tissue reagent solution (Qiagen) or RNA-later® reagent and shipped to the UCLA Brain Tumor Research Laboratory. RNA Seq will be performed at the UCLA GenoSeq Core facility and analyzed. We will assess the number of somatic mutations in each tumor and this data will be correlated with clinical variables to identify potential biomarkers with prognostic and predictive value for outcomes (ORR, PFS, OS). We will also develop immune signatures with the sequencing data and evaluate whether the presence of a pre-existing immune signature can also be correlated with clinical variables.

8.5 Sample Shipping Instructions & Details to UCLA

Guidelines for submission of archival tumor tissue (FFPE), fresh-preserved tumor tissue, and/or whole blood (please see Table 6 below for details):

- A memorandum indicating the study, the date of submission, name of study site submitting the tissue, and a list of contents. The DFCI Neuro-Oncology Coordinating Center or UCLA Brain Tumor Research lab will supply a template memorandum to sites at the time of the SIV or upon request.
- The DFCI Neuro-Oncology Coordinating Center will provide supplemental 16-225 Sample Collection & Shipping Guidance to sites at the time of activation or upon request.
- A copy of the pathology and surgical report for the sample being submitted should be included in the shipment.
- Slides should be shipped in a plastic slide holder/slide box. Place a small wad of padding in top of the container in order to avoid slides breaking during shipping and handling process.
- All blood & tissue samples addressed to UCLA should be shipped via FedEx for overnight

delivery. On-study tissue & blood samples should be obtained Sunday-Thursday for a Monday-Friday arrival at UCLA. **UCLA is unable to accept samples on Saturday or Sundays.**

- An email should be sent before or at the time of each shipment to the UCLA Brain Tumor Research lab below or the DFCI Neuro Oncology Coordinating Center at NeuroOnc_Coor@dfci.harvard.edu (or the designated Coordinating Center Project Manager) indicating what is being shipped and when.
- Please note that the submitting institution is responsible for the costs of shipping and handling.
- Ship samples to:

  Robert M. Prins, Ph.D.
  C/O Sylvia Odesa
  Gonda Research Lab, Room 1554
  695 Charles E. Young Drive South
  Los Angeles, CA 90095
  Telephone: 310-794-5663
  Email: RPrins@mednet.ucla.edu and SOdesa@mednet.ucla.edu

TABLE 6 CORRELATIVE SAMPLE SUMMARY

Table 6 Continued on next page

Biomarker name (Lead PI and Site)	Assay	Tissue/Body Fluid Tested and Timing of Assay	Collection and packaging	Address to send sample
TIL Density and TCR Overlap (R Prins/T Cloughesy/ L Liau, UCLA)	TCR ImmunoSeq and TCR Overlap (Adaptive Biotech.) on tumor tissue and peripheral blood mononuclear cell genomic DNA	Tumor (protocol surgery) and peripheral blood (Pre-Tx, Post-Tx)	200 mg of fresh tissue (approximately 20% tumor nuclei) sent in 2 mL Allprotect ® tissue reagent (Qiagen) or RNA-later® reagent Pre-treatment and post-treatment PBMC can be used from blood collected for flow cytometry (see below)	Robert M. Prins, Ph.D. C/O Sylvia Odesa Gonda Research Lab, Room 1554 695 Charles E. Young Drive South Los Angeles, CA 90095 Telephone: 310-794-5663 Email: RPrins@mednet.ucla.edu and SOdesa@mednet.ucla.edu
IHC measurements (R Prins/T Clougesy/L Liau, UCLA)	PD-1/PD-L1/CD8/CD4/CD8/Iba-1/CD68/GFAP IHC on FFPE tumor tissue	Archived and protocol surgery from tumor block	Archived: 1 formalin-fixed paraffin embedded (FFPE) tumor tissue block (preferred) from most recent surgery revealing glioblastoma Protocol Surgery: 1 FFPE tumor tissue block (preferred) or a minimum of 10 FFPE unstained sections	Robert M. Prins, Ph.D. C/O Sylvia Odesa Gonda Research Lab, Room 1554 695 Charles E. Young Drive South Los Angeles, CA 90095 Telephone: 310-794-5663 Email: RPrins@mednet.ucla.edu and SOdesa@mednet.ucla.edu
Peripheral blood T cell subsets/activation markers (R Prins/ T Cloughesy/ L Liau, UCLA)	Flow Cytometry on peripheral blood mononuclear cells	Peripheral blood (Pre-surgery pre-treatment, pre-surgery post treatment, with every MRI)	Pre-surgery pre treatment blood (for Groups A and B) : 10 Green top tubes (10ml/tube – sodium heparin tubes preferred) following registration/randomization and pre-pembrolizumab for Group A Pre-surgery, day 7 post pembrolizumab administration (+/- 2 days): 10 Green top tubes 10-ml sodium heparin tubes preferred (should not be same timepoint as next "pre-surgery post treatment" blood) Pre-surgery:10 Green top tubes 10ml – sodium heparin tubes preferred (should be greater than 10 days from initial dose of Pembrolizumab for Group A – Group B should also have a sample drawn pre-surgery, greater than 10 days from initial sample) Blood with each MRI (- 7 days before next cycle pembrolizumab infusion): 10 Green top tubes 10ml – sodium heparin tubes preferred)	Robert M. Prins, Ph.D. C/O Sylvia Odesa Gonda Research Lab, Room 1554 695 Charles E. Young Drive South Los Angeles, CA 90095 Telephone: 310-794-5663 Email: RPrins@mednet.ucla.edu and SOdesa@mednet.ucla.edu
Gene expression signatures and somatic mutations (R Prins / T Cloughesy / L Liau)	RNA Seq on tumor RNA	Tumor (protocol surgery) and peripheral blood	200 mg of tissue (approximately 20% tumor nuclei) sent in 2 mL Allprotect ® tissue reagent (Qiagen) or RNA-later® reagent	Robert M. Prins, Ph.D. C/O Sylvia Odesa Gonda Research Lab, Room 1554 695 Charles E. Young Drive South Los Angeles, CA 90095 Telephone: 310-794-5663 Email: RPrins@mednet.ucla.edu and SOdesa@mednet.ucla.edu

TABLE 6 CORRELATIVE SAMPLE SUMMARY (CONT'D)

Biomarker name (Lead PI and Site)	Assay	Tissue/Body Fluid Tested and Timing of Assay	Collection and packaging	Address to send sample
Tumor mRNA from extracellular vesicles (J Heath, Institute for Systems Biology / Prins Lab - processing)	Liquid biopsy of a partial tumor transcriptome	EDTA plasma (Pre-surgery pre-treatment, pre-surgery post treatment, with every MRI)	Pre-surgery pre-treatment blood: 2-10ml K2EDTA tubes following registration/randomization and pre-pembrolizumab Pre-surgery: 2-10ml K2EDTA tubes same day or -1 day from on-study surgery Blood with each MRI (- 7 days before next cycle pembrolizumab infusion): 2-10ml K2EDTA tubes Blood with each MRI (- 7 days before next cycle pembrolizumab infusion): 2-10ml K2EDTA tubes	Robert M. Prins, Ph.D. C/O Sylvia Odesa Gonda Research Lab, Room 1554 695 Charles E. Young Drive South Los Angeles, CA 90095 Telephone: 310-794-5663 Email: RPrins@mednet.ucla.edu and SOdesa@mednet.ucla.edu *Prins lab will subsequently process and ship to:* *James Heath, PhD* *C/O Rick Edmark* *Heath Lab* *Institute for Systems Biology* *401 Terry Ave.* *N. Seattle, WA 98109*

9. STUDY CALENDAR

Screening/baseline evaluations are to be conducted within 14 days of registration unless indicated otherwise. Assessments must be performed and reviewed prior to administration of any study agent at any treatment visit unless otherwise noted in footnotes below. Study assessments should be performed and study drug should be administered within +3/-3 days of the protocol-specified date, unless otherwise noted.

Assessments	Screening [a]	Pre-Surgery [b]	Pre-Surgery Day7 [ff]	Surgery	Post-Surgery	Post-Surgery Treatment Cycles D1 [b]	Post-Surgery Treatment Cycles D22 [c]	End of Tx [d]	30-Day Post Drug [e]	Active Follow Up [f]	Long Term Follow Up [g]
Informed Consent [h]	X										
Background Information/History [i]	X										
Inclusion/Exclusion Criteria [j]	X										
Vital Signs [k]	X	X				X	X	X			
Neurologic Exam [l]	X	X				X		X			
Directed Physical Exam [m]	X	X				X	X	X			
Karnofsky Performance Status [n]	X	X				X		X	X		
Concomitant Medications [o]	X		----------X [p]----------								
Adverse Event Assessment [p]			----------X [q]----------								
Pregnancy Test – Urine or Serum β-HCG [q]	X					X		X			
Coagulation [r]	X										
Hematology [s]	X	X				X	X	X			
Serum Chemistry [t]	X	X				X	X	X			
T3, FT4, TSH [u]	X	X				X		X			
Imaging – CT or MRI [v]	X				X [v,ee]	X [v]		X		X	
Response Assessment [w]						X		X		X	
Pembrolizumab Administration [x]		X [x]				X [x]	X				
Blood for Flow Cytometry [z]		X	X	X		X		X			
Blood for tumor mRNA [y]		X		X		X		X			
Tumor Resection [aa]				X							
Submission of Archival Tissue [bb]		X									
Post-End-of-Treatment Therapies [cc]									X	X	X
Survival [dd]									X	X	X

a. All screening procedures to be performed within 14 days of registration, except informed consent may occur up to 28 days prior to registration.
b. Pre-Surgery Day 1 and Day 1 of subsequent cycles: For Pre-Surgery Day 1 only, screening assessments may serve as day 1 assessments, except in the event that there are any indications that the participant's condition is deteriorating for which laboratory evaluations should be repeated within 48 hours prior to initiation of on-study assessments. For all subsequent cycles (post-surgery), required assessments should be performed within 3 days of scheduled cycle day 1.
c. Day 22 window requirements: pembrolizumab must be administered +/-3 days from Day 22 of each cycle. Assessments must be performed and reviewed prior to study agent administration.
d. End of Tx: End of treatment assessments to be performed within 7 days after last drug administration or within 7 days after decision to end treatment. Assessments may continue for ongoing reportable adverse events.
e. 30-Day Post Drug: A contact/visit is to be performed at 30 days (+ 7 days) after the last study drug is given. This may be performed via documented phone conversation with a study nurse or clinician or a clinic visit. All participants will be followed until resolution or stabilization of any serious or reportable adverse events occurring during treatment or starting within 30 days of last study drug.
f. Active Follow Up: For participants who discontinue study treatment for reasons other than disease progression, every effort should be made to continue monitoring their disease status by radiologic imaging at a schedule developed by the treating investigator until (1) documented disease progression, (2) death or (3) the end of the study, whichever occurs first.
g. Long Term Follow Up: participants will be followed every 3 months (+/- 1 month) via contact or medical record review until death for post-treatment therapies, reason for stopping those therapies and survival.
h. Informed Consent: Must be obtained by MD attending. No study specific screening procedures may occur until after the informed consent process is complete. Informed Consent may be obtained within 28 days of registration.
i. Background information/history: to include review of treatment history for GBM, any ongoing medical conditions and medical history pertaining to eligibility on study and involvement during study.
j. Inclusion/exclusion criteria: source documentation providing investigator's confirmation that the participant had met all eligibility criteria must be available prior to registration.
k. Vital signs: weight, heart rate, blood pressure, respiration rate, temperature. Vital signs must be performed prior to administration of treatment on treatment days. Height required only at screening and may be obtained within 1 year of registration.
l. Neurologic Exam: to be completed by the investigator or qualified designee at screening, C1D1 and start of all subsequent cycles.
m. Directed Physical Exam: to be completed as clinically indicated by the investigator or qualified designee.
n. Performance Status: See Appendix A for KPS scale.
o. Concomitant medications: concomitant medications and reason for administration should be documented in the case history from date of consent up to the 30-Day Post Drug Visit. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs.
p. Adverse event assessment: adverse events experienced by participants will be collected and recorded from registration to the 30-Day Post Drug Visit of the last dose of study medication (+ 7 days depending on when 30-Day Post Drug visit/contact occurs) and all SAEs (related and unrelated to trial treatment) / ECIs up to 90 days after the last dose of trial treatment or the start of new anti-cancer treatment, whichever comes first. Afterwards, report only SAEs and ECIs that are considered related to trial treatment. Adverse events may also occur in screened subjects during pre-allocation baseline period as a result of a protocol-specific intervention, including washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.
q. Pregnancy Test: For women of child bearing potential, a pregnancy test must be performed. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.
r. Coagulation: PT/INR, PT, PTT required at screening only and then as clinically indicated.
s. Hematology: erythrocytes (RBC), hemoglobin, hematocrit, platelets, total WBC plus differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils).
t. Serum Chemistry: albumin, alkaline phosphatase (ALP), bicarbonate (HCO_3) or CO_2, BUN, calcium, chloride, creatinine, glucose, magnesium, potassium, SGOT (AST), SGPT (ALT), sodium, total protein, total bilirubin.

u. T3,FT4, TSH: total triiodothyronine (T3), free thyroxine (T4), and thyroid stimulating hormone (TSH). T3, FT4, TSH must be drawn prior to pembrolizumab administration; if results are not available for review prior to pembrolizumab administration, study treatment may proceed. Results should be reviewed when they become available.
v. Imaging: gadolinium-enhanced contrast and non-contrast MRI. CT alternative, if MRI contraindicated. Initial imaging should be performed within 14 days prior to registration. On-study imaging should be performed every 6 weeks at Day 1 of each cycle (-7 days). The same imaging technique should be used in a participant throughout the trial, if feasible. Local reading (investigator assessment) will be used to determine eligibility and for participant management.
w. Response Assessment: Per RANO criteria (section 10).
x. Pembrolizumab Administration: Pre-surgery pembrolizumab required for participants randomized to Group A only (14 days +/-5 days prior to day of surgery). Post-surgery pembrolizumab must start no more than 35 days following surgery. Post surgery pembrolizumab may be administered +/- 3 days from days 1 and 22 of each cycle.
y. Blood for tumor mRNA: please see Table 6 Correlative Sample Summary for details on collection requirements.
z. Blood for Flow Cytometry: please see Table 6 Correlative Sample Summary for details on collection requirements.
aa. Tumor Resection: please see Table 6 Correlative Sample Summary for details on collection requirements. On-study surgery tissue should be submitted within 60 days of surgery.
bb. Submission of archival tissue: archival tumor tissue should be submitted within 60 days of registration. Please see section 8.3 for details.
cc. Post-end-of-treatment oncology therapies: start/stop dates, names of treatment regimens and reason for stopping should be collected.
dd. Survival: date of death and reason should be collected for overall survival purposes, when applicable.
ee. For post-surgery MRI (for both Group A and B participants), if the post-surgery MRI is greater than 21 days from post-surgery pembrolizumab initiation, then MRI must be repeated.
ff. Blood for Flow Cytometry (added in Stage 2) must be drawn 7 days after initial pembrolizumab administration (+/- 2 days). Please see Table 6 Correlative Summary for details on collection requirements.

10. MEASUREMENT OF EFFECT

Tumor response will be assessed every 6 weeks for patients treated on this study using RANO criteria[104] as outlined below. Clinicians may repeat response assessment more frequently as clinically indicated.

10.1 Anti-Tumor Effect Definitions

10.1.1 Evaluable for toxicity. All participants who receive at least one dose of study treatment will be evaluable for toxicity from the time of their first treatment.

10.1.2 Evaluable for objective response. Only those participants who have measurable disease present at baseline (obtained within 14 days of cycle 1, day 1) scan and have received at least one dose of therapy will be considered evaluable for response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression or die prior to the end of cycle 1 will also be considered evaluable.)

10.1.3 Measurable disease. Bi-dimensionally, contrast-enhancing, measurable lesions with clearly defined margins by CT or MRI scan, with a minimal diameter of 1 cm, and visible on 2 axial slices which are at least 5 mm apart with 0 mm skip. Measurement of tumor around a cyst or surgical cavity, if necessary, requires a minimum thickness of 3 mm. If there are too many measurable lesions to measure at each evaluation, the investigator must choose the largest two to be followed before a participant is entered on study. The remaining lesions will be considered non-measureable for the purpose of objective response determination. Unless progression is observed, objective response can only be determined when all measurable and non-measurable lesions are assessed.

10.1.4 Non-measurable evaluable disease. Unidimensionally measurable lesions, masses with margins not clearly defined, lesions with maximal diameter < 1cm.

10.2 Response/Progression Categories

10.2.1 Complete response (CR). All of the following criteria must be met:

a) Complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks. In the absence of a confirming scan 4 weeks later, this scan will be considered only stable disease.

b) No new lesions.

c) All measurable and non-measurable lesions must be assessed using the same techniques as baseline.

d) Participants must be on no steroids or on physiologic replacement doses only.

e) Stable or improved non-enhancing (T2/FLAIR) lesions

f) Stable or improved clinically, for clinical signs and symptoms present at baseline and recorded to be disease related

Participants with non-measurable disease cannot have a complete response. The best response possible is stable disease.

10.2.2 Partial response (PR). All of the following criteria must be met:

a) Greater than or equal to 50% decrease compared to baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks. In the absence of a confirming scan 4 weeks later, this scan will be considered only stable disease.

b) No progression of non-measurable disease.

c) No new lesions.

d) All measurable and non-measurable lesions must be assessed using the same techniques as baseline.

e) The steroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan.

f) Stable or improved non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared to baseline scan.

g) Stable or improved, for clinical signs and symptoms present at baseline and recorded to be disease related clinically.

Participants with non-measurable disease cannot have a partial response. The best response possible is stable disease.

10.2.3 Progressive disease (PD). Any of the following criterion must be met:

a) > 25% increase in sum of the products of perpendicular diameters of enhancing lesions (over best response or baseline if no decrease) on stable or increasing doses of corticosteroids

b) Any new enhancing measurable lesion

c) Clear clinical deterioration not attributable to other causes apart from the tumor (e.g. seizures, medication side effects, complications of therapy, cerebrovascular events, infection, etc.). The definition of clinical deterioration is left to the discretion of the investigator but it is recommended that a decline in the Karnofsky Performance Score (KPS) from 100 or 90 to 70 or less, a decline in KPS of at least 20 from 80 or less, or a decline in KPS from any baseline to 50 or less, for at least 7 days, be considered neurologic deterioration, unless attributable to co-morbid events or changes in corticosteroid dose.

d) Failure to return for evaluation due to death or deteriorating condition

10.2.4 Stable disease (SD). All of the following criteria must be met:

a) Does not qualify for CR, PR, or progression.

b) All measurable and non-measurable sites must be assessed using the same techniques as baseline.

c) Stable clinically.

10.2.5 Unknown response status. Progressive disease has not been documented and one or more measurable or non-measurable lesions have not been assessed.

The RANO Response Criteria to be used in this study are summarized in Table 7.

Table 7 Summary of the RANO Response Criteria

	CR	PR	SD	PD#
T1-Gd +	None	≥50% decrease	<50% decrease- <25% increase	≥25% increase*
T2/FLAIR	Stable or decrease	Stable or decrease	NA	NA
New Lesion	None	None	None	Present*
Corticosteroids	None	Stable or decrease	Stable or decrease	Stable or increasing
Clinical Status	Stable or increase	Stable or increase	Stable or increase	Decrease*
Requirement for Response	All	All	All	Any*

CR=complete response; PR=partial response; SD=stable disease; PD=progressive disease; NA= not applicable

#: Progression occurs when any of the criteria with * is present

Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration

Of note, patients who require increased corticosteroids within two weeks of MRI assessment (relative to the dose taken at the time of the prior assessment) cannot be classified as CR, PR or SD and should be classified as non-evaluable at that time point. Conversely, patients who decrease corticosteroids within two weeks of MRI assessment (relative to the dose taken at the time of the prior assessment) cannot be classified as PD and should be classified as non-evaluable.

10.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation, using a ruler, calipers, or digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 14 days before the beginning of the treatment.
The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up

10.4 Evaluation of Best Response

The best overall response is the best response recorded from the start of the treatment until disease progression (taking as reference for progressive disease the smallest measurements recorded since the treatment started). If a response recorded at one scheduled MRI does not persist at the next regular scheduled MRI, the response will still be recorded based on the prior scan, but will be designated as a non-sustained response. If the response is sustained, i.e. still present on the subsequent MRI at least four weeks later, it will be recorded as a sustained response, lasting until the time of tumor progression. Participants without measurable disease may only achieve SD or PD as their best "response."

10.5 Modified RANO (iRANO)[111]: Study Continuation Beyond Initial Progressive Disease

Immunotherapeutic agents such as pembrolizumab may produce antitumor effects by potentiating endogenous cancer-specific immune responses which may manifest as initial worsening of enhancement and edema on MRI or CT scans (i.e. pseudoprogression). In addition, the response patterns seen with immunotherapeutics may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest a clinical response after an initial increase in tumor burden or even the appearance of new lesions. For these reasons, the immune-related response criteria (irRC) have endorsed continuation of study therapy beyond initial radiographic evidence of progression for clinically stable patients undergoing immune based therapies.[105]

A major advance of the RANO criteria[104] to assess response in neuro-oncology over the previously used Macdonald criteria[106] includes recognition of the prevalence of pseudoprogression during the first three months following completion of radiation and daily temozolomide.[107,108] Specifically, RANO permits patients with such progressive MRI findings to continue temozolomide therapy for up to three months in order to avoid inaccurately classifying such patients as progressive. Furthermore, RANO permits patients with progressive radiographic findings at anytime to continue current therapy pending follow-up imaging if the etiology of progressive imaging findings is unclear. Standard RANO may not provide an accurate response assessment of immunotherapeutic agents such as pembrolizumab.

Therefore, the following adaptations of the RANO criteria will be used to assess response for patients treated on this study in an exploratory fashion (Table 8):

- Potential Pseudoprogression: If radiologic imaging shows initial PD, subjects who are not experiencing significant clinical decline (e.g. significant decrease in KPS see section 10.2.3), may be allowed to continue study treatment for up to three months. Patients should

be closely monitored with MRIs every cycle (approximately every 6 weeks) during this period. Patients who have radiographic evidence of further progression after up to three months, or who decline significantly at anytime, will be classified as progressive with the date of disease progression back-dated to the first date that the subject met criteria for progression and such subjects will be discontinued from study therapy. Although the kinetics of pseudoprogression due to immune checkpoint blockade among glioblastoma patients is currently unknown, three months is a reasonable estimate based on: 1) the peak time for XRT/daily temozolomide-related pseudoprogression is usually within three months of completion for glioblastoma patients[107,108] and; 2) three months is also the most common timeframe for pseudoprogression observed among patients with advanced melanoma or other solid tumors treated with PD-1/PD-L1immune checkpoint blockade to date.[66,69,109]

Among patients on this study with initial radiographic PD, tumor assessment should be repeated regularly (every cycle, approximately every 6 weeks) in order to confirm PD with the option of continuing treatment as described below while awaiting radiologic confirmation of progression. If repeat imaging shows a stabilization or reduction in the tumor burden compared to the initial scan demonstrating PD, treatment may be continued / resumed. If repeat imaging after up to three months confirms progressive disease, then the date of disease progression will be the first date the subject met criteria for progression and subjects will be discontinued from study therapy. Subjects who have confirmed disease progression will discontinue study medication and enter the follow up/survival phase of the study. In determining whether or not the tumor burden has increased or decreased, investigators should consider all target lesions as well as non-target lesions.

- Tumor Enhancement to Define Progression: RANO expanded the previously utilized Macdonald criteria[106] to include the development of "significantly" increased T2 or FLAIR abnormality in the definition of progressive disease because such changes can be a major component defining radiographic progression following therapeutic use of VEGF/VEGFR-targeting therapeutics which are known to elicit potent anti-permeability changes that limit contrast uptake. However, immune based therapies are expected to be associated with inflammatory changes that may include edema. Therefore, radiographic progressive disease will be defined by assessment of enhancing tumor and will not declare tumor progression based on the presence of T2 or FLAIR changes alone as outlined in RANO because:
  - There is no expectation that immunotherapy agents including PD-1 inhibitors will falsely diminish enhancing tumor burden as has been noted with anti-angiogenic therapies; and
  - Immune based therapies are expected to induce inflammatory responses which may be associated with increased edema and T2/FLAIR changes. Such radiographic finding may inaccurately be interpreted to represent tumor progression (i.e. pseudoprogression).

In subjects who have initial evidence of radiographic PD, it is at the discretion of the treating physician whether to continue a subject on study treatment for up to three months pending

confirmation of PD on follow-up imaging. This clinical judgment decision should be based on the subject's overall clinical condition, including performance status, clinical symptoms, and laboratory data. Subjects may receive study treatment while waiting for confirmation of PD if they are not experiencing significant clinical decline and the subject is adequately tolerating study therapy (if a subject is required to discontinue study treatment for toxicity as defined per protocol section 5, then they must be taken off-treatment).

When feasible, study therapy should not be discontinued until radiographic progression is confirmed. This allowance to continue treatment despite initial radiologic progression takes into account the observation that some subjects can have a transient tumor flare in the first few months after the start of immunotherapy, but with subsequent disease response.[105] Subjects that are exhibiting significant neurologic decline are not required to have repeat imaging for confirmation of progressive disease.

Table 8 Imaging and Treatment After 1st Radiologic Evidence of PD

	No Significant Neurologic Decline		Significant Neurologic Decline	
	Imaging	Treatment	Imaging	Treatment
1st radiologic evidence of PD	Repeat imaging (every cycle, approximately every 6 weeks) for up to 3 months to confirm PD	May continue study treatment at the Investigator's discretion for up to 3 months while awaiting confirmatory scans	Repeat imaging >6 weeks later to confirm PD if possible	Discontinue treatment
Repeat scan up to 3 months after 1st radiologic evidence confirms PD	No additional imaging required; date of tumor progression back-dated to date of initial radiographic PD	Discontinue treatment	No additional imaging required	Not applicable
Repeat scan shows SD, PR or CR	Continue regularly scheduled imaging assessments every 6 weeks	Continue study treatment at the Investigator's discretion	Continue regularly scheduled imaging assessments every 6 weeks	May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion

Participants with progressive radiographic findings are encouraged to undergo surgical intervention in order to delineate pseudoprogression due to inflammation associated with PD-1 blockade from true tumor progression. Participants with histopathologic findings of significant immune infiltrate and evolving gliosis will be allowed to continue study therapy. In contrast, those with clear evidence of progressive tumor by histopathologic evaluation will be defined as progressive and discontinued from study therapy. For such patients, the date of tumor progression will be the first date the participant met radiographic criteria for PD

10.6 Central Radiology Review

The central review of neuroimaging (MRI or CT) will be performed at Dana- Farber Cancer Institute or UCLA (as determined by Overall PI) on participants when requested from the DFCI Coordinating Center on behalf of the Overall PI. All films of all views from pre-registration (including pre-enrollment scans documenting progression prior to study interventions) and subsequent scans (including all on-study and follow-up scans) will be requested for central review. CDs are preferred.

A copy of all scan reports should be attached for inclusion in the submission. Once the Central Review is complete the Reviewing Physician will document the review results. Once the Central Review is complete, the central review results may be made available to the local PI or treating investigator.

When requested from the DFCI Coordinating Center, please send a copy of all scans to:

Dr. Patrick Wen c/o Myriam Bednarek Debruyne
Center for Neuro-Oncology
Dana-Farber Cancer Institute
450 Brookline Ave
Boston, MA 02215
ph: 617-582-9314
fax: 617-582-7782
NeuroOnc_Coord@dfci.harvard.edu

A memo must be submitted to the DFCI Coordinating Center each time submissions are made including DFCI study number, participant identifiers and details of what is being submitted.

The submitting institution is responsible for the costs of shipping and handling.

11. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

11.1 Data Reporting

11.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

11.2 Responsibility for Data Submission

All investigative sites are responsible for submitting data and/or data forms to the Office of Data Quality (ODQ) in accordance with DF/HCC policies.

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration with ODQ
On Study Form	Within 30 days of registration
Baseline Assessment Form	Within 30 days of registration
Treatment Form	Within 14 days of treatment administration
Adverse Event Report Form	Within 14 days of AE assessment/notification
Response Assessment Form	Within 14 days of the response assessment
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

11.3 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of medical oncologists, research nurses, pharmacists and biostatisticians with direct experience in cancer clinical research. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year with the frequency determined by the outcome of previous reviews.. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days

of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

11.4 Multicenter Guidelines

This protocol will adhere to the policies and requirements of the DF/HCC Multi-Center Data and Safety Monitoring Plan (Appendix B). The specific responsibilities of the Overall PI, Coordinating Center, and Participating Institutions and the procedures for auditing are presented in Appendix B.

- The Overall PI/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.

- Mechanisms will be in place to ensure quality assurance, protocol compliance, and adverse event reporting at each site.

- Except in very unusual circumstances, each participating institution will order the study agent(s) directly from supplier. A participating site may order the agent(s) only after the initial IRB approval for the site has been forwarded to the Coordinating Center.

12. STATISTICAL ANALYSIS PLAN

This section outlines the statistical analysis strategy and procedures for the study. If, after the study has begun, changes are made to primary and/or key secondary hypotheses, or the statistical methods related to those hypotheses, then the protocol will be amended (consistent with ICH Guideline E-9). Changes to exploratory or other non-confirmatory analyses made after the protocol has been finalized, along with an explanation as to when and why they occurred, will be listed in the Clinical Study Report (CSR) for the study. Post hoc exploratory analyses will be clearly identified in the CSR.

12.1 Statistical Analysis Plan Summary

This section contains a brief summary of the statistical analyses for this trial.

Stage 1:
The primary outcome, tumor infiltrating T lymphocyte (TIL) density, will be assessed by comparing Group A versus Group B.

Stage 2:
The primary outcome, cell cycle and cancer proliferation genetic signature, will be assessed by comparing Group A versus Group B.

Stage 1 and stage 2:
The secondary endpoint, PFS6, will be assess using pooled Group A and Group B patients and compared to appropriate historical controls.

12.1.1 Efficacy Analyses

The primary and key secondary endpoints, primary analysis population, and statistical methods that will be employed for the efficacy analyses are discussed in detail in the following sections.

12.1.2 Safety Analyses

Adverse events will be graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. All patients who receive any amount of Pembrolizumab will be evaluable for toxicity. The All-Patients-as-Treated population will be employed for safety analyses.

12.2 Statistical Analysis Plan

12.2.1 Responsibility for Analyses

The statistical analysis of the data obtained from this study will be the responsibility of the study responsible biostatistician.

This trial is being conducted as an open-label study, i.e., subjects, investigators, and SPONSOR personnel will be aware of subject treatment assignments after each subject is enrolled and treatment is assigned.

12.2.2 Hypotheses/Estimation

Objectives and hypotheses of the study are stated in Section 1.0.

12.2.3 Power and Sample Size for primary endpoint

Stage 1:
Group B is an independent concurrent control to evaluate TIL density in Group A due to the infeasibility of multiple resections within a short time period for patients with recurrent GBM. Patients will be randomly assigned to either group A or B with a 1:1 randomization ratio prior to surgery. Based on our preliminary data, the mean of TIL density is estimated to be 0.4 (T cell per nucleating) (SD = 0.5) in the control group (Group B). Fifteen patients per group (total of 30 evaluable) achieve 85% power to detect an increase of 0.5 of TIL density comparing Group A versus Group B at an alpha level of 0.05 (1-sided) using a two-sample t-test.

Stage 2:
An extension based on interim data has the primary scientific endpoint of evaluating the cell cycle signature in patients receiving neoadjuvant pembrolizumab. This extension will aim to enroll 25 additional patients with a goal of 20 evaluable patients to Group A. Group B is an independent

concurrent control to evaluate the cell cycle-related gene signature in Group A. Based on the preliminary data, the mean tumor cell cycle-related gene expression signature is estimated to be -0.3 for neoadjuvant pembrolizumab (SD=0.66) and +0.37 for control (SD=0.64), which corresponds to an effect size of 1.1. With the addition of 20 more patients in Group A, the one-sided two-sample T-test (alpha level: 0.05) will have 81.73% power to detect an effect size of 1.1 for the difference of tumor cell cycle-related gene expression signature between the two groups.

12.2.4 Analysis Endpoints

Efficacy and safety endpoints that will be evaluated for within- and/or between-treatment differences are listed below, followed by the descriptions of the derivations of selected endpoints

12.2.4.1 Efficacy Endpoints

Efficacy will be measured by percent PFS6 as defined by RANO.

12.2.4.2 Safety Endpoints

The primary safety endpoints are AEs graded using CTCAE (Version 5.0) criteria. Safety will be assessed by quantifying the toxicities and grades experienced by subjects who have received Pembrolizumab, including serious adverse events (SAEs) and events of clinical interest (ECIs). Other safety endpoints include laboratory safety assessments, KPS status, vital signs and physical examinations.

12.2.5 Analysis Population

12.2.5.1 Efficacy Analysis Populations

The Full Analysis Set (FAS) population will serve as the primary population for the analysis of efficacy data in this study. The FAS population consists of all subjects within each cohort who have received at least one dose of study treatment.

Two supportive analyses of the primary and selected secondary efficacy endpoints will be conducted. The first supportive analysis will be conducted in the FAS-2 population, defined as all subjects who meet the FAS population definition and have a post baseline scan OR discontinue the trial due to progressive disease/drug related AE. The second analysis will be conducted using the intention to treat (ITT) population, defined as all randomized subjects.

Subjects will be included in the cohort to which they are randomized for the analysis of efficacy data.

12.2.5.2 Safety Analysis Populations

The All Patients as Treated (APaT) population will be used for the analysis of safety data in this study. The APaT population consists of all allocated subjects who received at least one dose of study treatment.

At least one laboratory or vital sign measurement obtained subsequent to at least one dose of study treatment is required for inclusion in the analysis of each specific parameter. To assess change from baseline, a baseline measurement is also required.

Details on the approach to handling missing data for safety analyses are provided in Section 12.2.6 Statistical Methods.

12.2.6 Statistical Methods

Nominal p –values may be computed for efficacy analyses as a measure of strength of association between the endpoint and the treatment effect rather than formal tests of hypotheses. Unless otherwise stated, all statistical tests will be conducted at the α=0.05 (2-sided) level.

12.2.6.1 Statistical Methods for Primary Endpoint and Efficacy Analyses

Stage 1:
A two-sample Student's t-test will be used to test the difference of TIL density between the two groups. Data distribution will be examined prior to analysis. Data transformations will be performed as deemed appropriate.

Stage 2:
A two-sample Student's t-test will be used to test the difference of cell cycle-related signature between the two groups. Data distribution will be examined prior to analysis. Data transformations will be performed as deemed appropriate.

For PFS endpoint, the analyses will be conducted using pooled Group A and Group B patients. Kaplan-Meier (KM) curves and median estimates from the KM curves will be provided as appropriate. Percent PFS6 will be estimated from the KM curves and compared to historical controls. Subjects without efficacy evaluation data or without survival data will be censored at Day 1.

12.2.6.2 Statistical Methods for Safety Analyses

Safety and tolerability will be assessed by clinical review of all relevant parameters including adverse experiences (AEs), laboratory tests, and vital signs. Safety summaries will be reported for both cohorts.

Immune related adverse experiences (irAEs, as defined in Section 7.3.2) are prespecified as events of interest. These events will be summarized in separate tables from other AEs by toxicity grade and will include the counts, percentage, and 95% CI. Any AE of unknown etiology associated with pembrolizumab exposure will be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (irECI). Other ECIs listed in section 7.3.2 will also be summarized in the same manner as irAEs.

Adverse experiences (specific terms as well as system organ class terms) and predefined limits of

change in laboratory, and vital sign parameters that are not pre-specified as events of interest will be summarized with descriptive statistics (counts, percentage, mean, standard deviation, etc.).

Continuous measures such as changes from baseline in laboratory, and vital signs parameters that are not pre-specified as events of interest will be summarized using descriptive statistics (mean, standard deviation, etc.) for baseline, on-treatment, and change from baseline values.

12.2.6.3 Statistical Methods for Exploratory Analyses

Variables involved in exploratory analyses will be examined graphically and summarized by descriptive statistics. Data transformation may be applied to quantitative variables prior to analyses if deemed necessary.

Bivariate association analyses will be conducted to evaluate the associations between exploratory biomarkers and clinical outcomes and adverse events for the objectives listed in 1.4. The analyses will be performed for each group separately and/or combined as deemed appropriate. Specifically, Pearson or Kendall's tau correlation coefficients will be calculated to evaluate the association between quantitative variables, Chi-squared tests or Fisher's exact tests will be used to examine the association between categorical or ordinal variables, and ANOVA will be performed to study the association between a quantitative variable and a categorical variable. The association between biomarkers and clinical outcomes (PFS and OS) will be evaluated using Cox regression. For markers with multiple measurements over time, data at a specific time point or change from pre-treatment will be used in the above bivariate analyses as deemed appropriate.
Changes in markers pre- and post- treatment will be assessed using paired t-tests.

Between-group comparisons will be conducted for PFS, OS, and PD-1, PDL-1 IHC expression. Survival analyses via Kaplan-Meier curves and log-rank tests will be used to estimate and compare PFS, second PFS, and OS between Group A and Group B. PD-1 and PDL-1 IHC expression will be compared between Groups A and B using two-sample t-tests.

12.2.7 Summary of Baseline Characteristics and Demographics

Baseline characteristics will be assessed by the use of tables and/or graphs for each cohort separately. No statistical hypothesis tests will be performed on these characteristics. The number and percentage of subjects screened, randomized, the primary reasons for screening failure, and the primary reason for discontinuation will be displayed. Demographic variables (e.g., age, gender), baseline characteristics, primary and secondary diagnoses, and prior and concomitant therapies will be summarized by treatment either by descriptive statistics or categorical tables.

12.2.8 Compliance (Medication Adherence)

A day within the study will be considered an On-Therapy day if the subject receives the study medication infusion. The number of Days Should be on Therapy is the total number of days from the first day of study medication to the date of the last dose of study medication. For each subject, percent compliance will then be calculated using the following formula:

Percent Compliance =
(Number of Days Should be on Therapy/Number of Days on Therapy) x 100

Summary statistics will be provided on percent compliance by treatment group for the FAS population.

12.2.9 Extent of Exposure

Extent of Exposure for a subject is defined as number of cycles in which the subject receives the study medication infusion. Summary statistics will be provided on Extent of Exposure for APaT population.

13. PUBLICATION PLAN

This trial is intended for publication, even if terminated prematurely. Publication may include any or all of the following: posting of a synopsis online, abstract and/or presentation at a scientific conference, or publication of a full manuscript. The Sponsor will work with the authors to submit a manuscript describing trial results within 12 months after the last data become available, which may take up to several months after the last subject visit in some cases. The Overall Principal Investigator will post a synopsis of trial results for approved products on www.clinicaltrials.gov by 12 months after the last subject's last visit for the primary outcome, 12 months after the decision to discontinue development, or product marketing (dispensed, administered, delivered or promoted), whichever is later.

These timelines may be extended for products that are not yet marketed, if additional time is needed for analysis, to protect intellectual property, or to comply with confidentiality agreements with other parties. Authors of the primary results manuscript will be provided the complete results from the Clinical Study Report, subject to the confidentiality agreement. When a manuscript is submitted to a biomedical journal, the Sponsor's policy is to also include the protocol and statistical analysis plan to facilitate the peer and editorial review of the manuscript. If the manuscript is subsequently accepted for publication, the Sponsor will allow the journal, if it so desires, to post on its website the key sections of the protocol that are relevant to evaluating the trial, specifically those sections describing the trial objectives and hypotheses, the subject inclusion and exclusion criteria, the trial design and procedures, the efficacy and safety measures, the statistical analysis plan, and any amendments relating to those sections. The Sponsor reserves the right to redact proprietary information.

For multicenter trials, subsequent to the multicenter publication (or after public disclosure of the results online at www.clinicaltrials.gov if a multicenter manuscript is not planned), an investigator and his/her colleagues may publish their data independently. In most cases, publication of individual trial site data does not add value to complete multicenter results, due to statistical concerns. In rare cases, publication of single trial site data prior to the main paper may be of value. Limitations of single trial site observations in a multicenter trial should always be described in such a manuscript.

Authorship credit should be based on 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. Authors must meet conditions 1, 2 and 3. Significant contributions to trial execution may also be taken into account to determine authorship, provided that contributions have also been made to all three of the preceding authorship criteria. Although publication planning may begin before conducting the trial, final decisions on authorship and the order of authors' names will be made based on participation and actual contributions to the trial and writing, as discussed above. The first author is responsible for defending the integrity of the data, method(s) of data analysis and the scientific content of the manuscript.

The Sponsor must have the opportunity to review all proposed abstracts, manuscripts or presentations regarding this trial 45 days prior to submission for publication/presentation. Any information identified by the Sponsor as confidential must be deleted prior to submission; this confidentiality does not include efficacy and safety results. Sponsor review can be expedited to meet publication timelines.

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APPENDIX A KARNOFSKY PERFORMANCE STATUS

Grade	Description
100	Normal, no complaints; no evidence of disease
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self; unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his personal needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled; requires special care and assistance.
30	Severely disabled; hospital admission is indicated although death not imminent.
20	Very sick; hospital admission necessary; active supportive treatment necessary.
10	Moribund; fatal processes progressing rapidly.
0	Dead.

* Schag CC, Heinrich RL, Ganz PA. Karnofsky performance status revisited: Reliability, validity and guidelines. J Clin Oncol 2:187-193, 1984.

APPENDIX B MULTI-CENTER DSMP

DFCI IRB Protocol #: **16-225**

Dana-Farber/Harvard Cancer Center
Multi-Center Data and Safety Monitoring Plan

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1.0 INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for conducting a DF/HCC Multi-Center research protocol.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center Multi-Center protocol will comply with Federal Regulations, Health Insurance Portability and Accountability Act (HIPAA) requirements and applicable DF/HCC Standard Operating Procedures.

1.2 Multi-Center Data and Safety Monitoring Plan Definitions

DF/HCC Multi-Center Protocol: A research protocol in which one or more outside institutions are collaborating with Dana-Farber/Harvard Cancer Center where a DF/HCC investigator is the sponsor. DF/HCC includes Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates.

Lead Institution: Among the Dana-Farber/Harvard Cancer Center sites (DFCI, MGH, BIDMC, CHB, BWH), the Dana-Farber Cancer Institute will be the Lead Institution and will be responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and applicable regulatory guidelines (FDA, etc.). The Lead Institution is the home of the Overall PI.

DF/HCC Sponsor: The person sponsoring the submitted Multi-Center protocol who takes responsibility for initiation, management and conduct of the protocol at all research locations. The DF/HCC Sponsor has ultimate authority over the protocol and is responsible for the conduct of the study at DF/HCC and all Participating Institutions. For this protocol the DF/HCC Sponsor is the same person as the DF/HCC Overall Principal Investigator.

Participating Institution: An institution that desires to collaborate with DF/HCC and commits to accruing participants to the DF/HCC protocol. The Participating Institution acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.

Coordinating Center: The entity that provides administrative support to the DF/HCC Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as specified in applicable regulatory guidelines. In general, the Lead Institution is the Coordinating Center for the DF/HCC Multi-Center Protocol.

DF/HCC Office of Data Quality: A group within DF/HCC responsible for registering human subjects for trials, ensuring high-quality standards are used for data collection and the ongoing management of clinical trials, auditing, and data and safety monitoring. ODQ also coordinates quality assurance efforts related to multi-center clinical research.
DF/HCC Clinical Trials Research Informatics Office (CTRIO): A group within DF/HCC responsible for providing comprehensive data management platform for managing clinical trial data.

2.0 GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the DF/HCC Sponsor, the Coordinating Center, and the Participating Institutions are expected to adhere to the following general responsibilities:

2.1 DF/HCC Sponsor

The DF/HCC Sponsor, Patrick Y. Wen, MD, will accept responsibility for all aspects of conducting a DF/HCC Multi-Center protocol which includes but is not limited to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Ensure that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol.
- Include the Multi-Center Data and Safety Monitoring Plan as an appendix to the protocol.
- Ensure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team member receives adequate protocol training (and/or a Site Initiation Visit prior to enrolling participants) and throughout trial's conduct as needed.
- Ensure the protocol will be provided to each participating site in a language understandable to all applicable site personnel when English is not the primary language.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Ensure all DFCI Institutional Review Board (IRB), DF/HCC and other applicable (i.e. FDA) reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with FDA (investigator-held IND trials), as applicable.
- Ensure compliance with all requirements as set forth in the Code of Federal Regulations, applicable DF/HCC requirements, HIPAA requirements, and the approved protocol.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the DF/HCC Sponsor.
- Identify and qualify Participating Institutions and obtain accrual commitments prior to extending the protocol to that site.
- Monitor accrual and address Participating Institutions that are not meeting their accrual requirements.

2.2 Coordinating Center

The general responsibilities of the Coordinating Center may include but are not limited to:

- Assist in protocol development
- Maintain FDA correspondence, as applicable.
- Review registration materials for eligibility and register participants from Participating Institutions in the DF/HCC clinical trial management system (CTMS).
- Distribute protocol and informed consent document updates to Participating Institutions as needed.
- Oversee the data collection process from Participating Institutions.
- Maintain documentation of Serious Adverse Event (SAE) reports and deviations/violations submitted by Participating Institutions and provide to the DF/HCC Sponsor for timely review and submission to the DFCI IRB, as necessary.

- Distribute serious adverse events reported to the DF/HCC Sponsor that fall under the DFCI IRB Adverse Event Reporting Policy to all Participating Institutions.
- Provide Participating Institutions with information regarding DF/HCC requirements that they will be expected to comply with.
- Carry out plan to monitor Participating Institutions either by on-site or virtual monitoring.
- Maintain Regulatory documents of all Participating Institutions which includes but is not limited to the following: local IRB approvals/notifications from all Participating Institutions, confirmation of Federal Wide Assurances (FWAs) for all sites, all SAE submissions, Screening Logs for all sites, IRB approved consents for all sites.
- Conduct regular communications with all Participating Institutions (conference calls, emails, etc) and maintain documentation of all relevant communications.

2.3 Participating Institution

Each Participating Institution is expected to comply with all applicable federal regulations and DF/HCC requirements, the protocol and HIPAA requirements.

The general responsibilities for each Participating Institution may include but are not limited to:

- Document the delegation of research specific activities to study personnel.
- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their local IRB.
- Maintain regulatory files as per sponsor requirements.
- Provide the Coordinating Center with regulatory documents or source documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as required (i.e. teleconferences).
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center prior to beginning research related activities.
- Submit Serious Adverse Event (SAE) reports to local IRB per institutional requirements and to the Coordinating Center, in accordance with DF/HCC requirements.
- Submit protocol deviations and violations to local IRB per institutional requirements and to the DF/HCC Sponsor in accordance with DF/HCC requirements.
- Order, store and dispense investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Have office space, office equipment, and internet access that meet HIPAA standards.
- Participate in any quality assurance activities and meet with monitors or auditors at the conclusion of a visit to review findings.
- Promptly provide follow-up and/or corrective action plans for any monitoring queries or audit findings.

3.0 DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

The following section will clarify DF/HCC Requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

3.1 Protocol Distribution

The Coordinating Center will distribute the final DFCI IRB approved protocol and any subsequent amended protocols to all Participating Institutions.

3.2 Protocol Revisions and Closures

The Participating Institutions will receive notification of protocol revisions and closures from the Coordinating Center. It is the individual Participating Institution's responsibility to notify its IRB of these revisions.

- **Non life-threatening revisions:** Participating Institutions will receive written notification of protocol revisions regarding non life-threatening events from the Coordinating Center. Non-life-threatening protocol revisions must be IRB approved and implemented within 90 days from receipt of the notification.

- **Revisions for life-threatening causes:** Participating Institutions will receive immediate notification from the Coordinating Center concerning protocol revisions required to protect lives with follow-up by fax, mail, e-mail, etc. Life-threatening protocol revisions will be implemented immediately followed by IRB request for approval.

- **Protocol closures and temporary holds:** Participating Institutions will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the Participating Institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

3.3 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for Participating Institutions. The Participating Institution consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for PI-Initiated Multi-Center Protocols. This document will be provided separately to each Participating Institution upon request.

Participating Institutions are to send their version of the informed consent document and

HIPAA authorization, if a separate document, to the Coordinating Center for review and approval prior to submission to their local IRB. The approved consent form must also be submitted to the Coordinating Center after approval by the local IRB for all consent versions.

The Principal Investigator (PI) at each Participating Institution will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. Participating institutions must follow the DF/HCC requirement that only attending physicians obtain informed consent and re-consent for all interventional drug, biologic, or device research.

3.4 IRB Documentation

The following must be on file with the Coordinating Center:

- Initial approval letter of the Participating Institution's IRB
- Copy of the Informed Consent Form(s) approved by the Participating Institution's IRB
- Participating Institution's IRB approval for all amendments
- Annual approval letters by the Participating Institution's IRB

3.5 IRB Re-Approval

Verification of IRB re-approval from the Participating Institutions is required in order to continue research activities. There is no grace period for continuing approvals.

The Coordinating Center will not register participants if a re-approval letter is not received from the Participating Institution on or before the anniversary of the previous approval date.

3.6 Participant Confidentiality and Authorization Statement

In 1996, congress passed the first federal law covering the privacy of health information known as the Health Insurance Portability and Accountability Act (HIPAA). Any information, related to the physical or mental health of an individual is called Protected Health Information (PHI). HIPAA outlines how and under what circumstances PHI can be used or disclosed.
In order for covered entities to use or disclose protected health information during the course of a study, the study participant must sign an authorization statement. This authorization statement may or may not be separate from the informed consent document. The Coordinating Center, with the approval from the DFCI IRB, will provide a consent template, with information regarding authorization for the disclosure of protected health information.

The DF/HCC Sponsor will use all efforts to limit its use of protected health information in its trials. However, because of the nature of these trials, certain protected health information must be collected. DF/HCC has chosen to use authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

3.6.1 DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center should be de-identified. It is recommended that the assigned protocol case number (as described below). Participant initials may be included or retained for cross verification of identification

3.7 DF/HCC Multi-Center Protocol Registration Policy

Eligible participants will be registered onto trial with the DF/HCC Office of Data Quality (ODQ) central registration system (by a Coordinating Center specialist, if participant is at a non-DF/HCC site). Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

A qualified member of the study team will confirm eligibility criteria and complete the protocol-specific eligibility checklist.

Issues that would cause treatment delays should be discussed with the Principal Investigator. If a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. Notify the ODQ Registrar (a Coordinating Center specialist, if participant is at a non-DF/HCC site) of participant status changes as soon as possible.

In order to register a participant onto study, the following must be done:

- Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.
- Complete the protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical/research record. **To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist.**

  Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.

Treatment may not begin without confirmation from the Coordinating Center that the participant has been registered.

Randomization can only occur during ODQ's normal business hours, Monday through Friday from 8:00 AM to 5:00 PM Eastern Standard Time.

3.7.1 Participant Registration and Randomization at a non-DF/HCC Site

To register a participant at any non-DF/HCC site, the subsequent procedure is to be followed:

1. The participating site's data manager/coordinator/research nurse should contact the lead-site Designee (Multi-Center Coordinating Center specialist) via telephone or email to:
   - Notify regarding the pending registration
   - Confirm the methods of sending documents and communication for registration
   - Communicate desired timeline of the registration (i.e. within the hour, the next day).

   Multi-Center DFCI Neuro-Oncology Designee contact information:

   Phone: 617-582-7101

   E-mail: NeuroOnc_Coor@dfci.harvard.edu

2. The data manager/coordinator/research nurse should then send the following documents to the Coordinating Center specialist:
   - Completed DF/HCC study specific Eligibility Screening Worksheet
   - Copy of protocol required test results (e.g. coagulation studies, hematology panel, serum pregnancy test, serum chemistry panel, urinalysis -- all as applicable per protocol)
   - Copy of the pathology and surgical reports
   - List of current concomitant medications (obtained within the protocol-specified screening window) including sign/date by RN/other clinician and documentation of when reviewed/confirmed with patient
   - Copy of signed informed consent form
   - Copy of signed HIPAA authorization form (if separate from the informed consent document)
   - Copy of clinic note(s) and other medical records that document consenting process, screening and eligibility, if available***

Documents will be transmitted via one of the following methods:
- *Scanned and emailed to: NeuroOnc_Coor@dfci.harvard.edu or direct email of Coordinating Center specialist*
- *Faxed to: 617-394-2683*

**** The Coordinating Center Specialists would like to review and monitor participant eligibility, informed consent, screening and baseline assessments on all participants. Providing a complete set of source documents prior to registration may delay registration. Participating Institutions will work with the Coordinating Center Specialists to determine what documents may feasibly be available for review prior to*

enrollment, and these documents are to be provided for pre-enrollment review. A complete set of documents will be provided to the Coordinating Center after registration; the timeline will be determined by the Coordinating Center Specialist based on the study team's experience with the trial and prior monitoring findings. If there are persistent issues with eligibility at a site or with a study overall, the Coordinating Center may require that all source documentation relevant to participant eligibility be provided prior to proceeding with participant registration.

3. After having received all transferred documentation, the Designee (Coordinating Center specialist) will review the documents to verify eligibility, and notify the participating site of the result.
4. The Designee (Multi-Center Coordinating Center specialist) will register the participant with ODQ Registrar (who validates eligibility and registers the participant onto study), and subsequently inform the participating site of the successful registration via Fax or email, to include:
    - Participant case number
    - Applicable Dose Treatment level and treatment arm assignment
5. The Designee (Multi-Center Coordinating Center specialist) will follow-up to confirm registration.

3.7.2 Initiation of Therapy

Participants must be registered with the DF/HCC ODQ before the initiation of treatment or other protocol-specific interventions. Treatment and other protocol-specific interventions may not be initiated until the Participating Institution receives confirmation of the participant's registration from the Coordinating Center. Therapy must be initiated per protocol guidelines. The DF/HCC Sponsor and DFCI IRB must be notified of any violations to this policy.

3.7.3 Eligibility Exceptions

No exceptions to the eligibility requirements for a protocol without DFCI IRB approval will be permitted. All Participating Institutions are required to fully comply with this requirement. The process for requesting an eligibility exception is defined below.

3.8 DF/HCC Protocol Case Number

At the time of registration, the following identifiers are required for all subjects: initials, date of birth, gender, race and ethnicity. Once eligibility has been established and the participant successfully registered, the participant is assigned a unique protocol case number. Participating Institutions should submit all de-identified subsequent

communication and documents to the Coordinating Center, using this case number to identify the subject.

3.9 Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity to ensure that researchers do not initiate changes in approved research without IRB review and approval, except when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor, who in turn is responsible for reporting to the DFCI IRB.

For reporting purposes, DF/HCC uses the terms "violation", "deviation" and "exception" to describe departures from a protocol. All Participating Institutions must adhere to these requirements for reporting to the DF/HCC Sponsor and will follow their institutional policy for reporting to their local IRB.

3.9.1 Definitions

Protocol Deviation: Any departure from the defined procedures set forth in the IRB-approved protocol which is *prospectively approved* prior to its implementation.

Protocol Exception: Any protocol deviation that relates to the eligibility criteria, e.g. enrollment of a participant who does not meet all inclusion/exclusion criteria.

Protocol Violation: Any protocol departure that was not *prospectively approved* by the IRB prior to its initiation or implementation.

3.9.2 Reporting Procedures

DF/HCC Sponsor: is responsible for ensuring that clear documentation is available in the medical record and/or regulatory documents to describe all protocol exceptions, deviations and violations. The DF/HCC Sponsor will also be responsible for ensuring that all protocol violations/deviations are promptly reported per DFCI IRB guidelines.

Participating Institutions: Protocol deviations require prospective approval from the DFCI IRB. The Participating Institution must submit the deviation request to the Coordinating Center who will then submit the deviation request to the Overall PI and DFCI IRB. Upon DFCI IRB approval the deviation is submitted to the Participating Institution IRB, per institutional policy. A copy of the Participating Institution's IRB report and determination will be forwarded to the Coordinating Center within 10 business days after the original submission. The deviation may not be implemented without required approvals.

Protocol violations occurring at a Participating Institution will be submitted to that site's own IRB per the IRB's reporting policy. Whether or not a violation needs to be reported to the local IRB, notification to the Coordinating Center of any violation should occur in a

timely manner. The Coordinating Center will provide training for the requirements for the reporting of violations.

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the Coordinating Center will submit the report to the DF/HCC Sponsor for review. Subsequently, the Participating Institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI IRB reporting guidelines.

3.10 Safety Assessments and Toxicity Monitoring

The study teams at all participating institutions are responsible for protecting the safety, rights and well-being of study participants. Recording and reporting of adverse events that occur during the course of a study help ensure the continuing safety of study participants.

All participants receiving investigational agents and/or other protocol mandated therapy will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported by participants. All toxicities encountered during the study will be evaluated according to the NCI criteria specified in the protocol. Life-threatening toxicities must be reported immediately to the DF/HCC Sponsor via the Coordinating Center and IRB, both DFCI and local as applicable.

Additional safety assessments and toxicity monitoring will be outlined in the protocol.

3.10.1 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Adverse Events (AEs) and Serious Adverse Events (SAEs) are detailed in protocol section 6.
Participating Institutions must report the SAEs to the DF/HCC Sponsor and the Coordinating Center following the DFCI IRB Advert Event Reporting Policy.

The Coordinating Center will maintain documentation of all Participating Institution Adverse Event reports and be responsible for communicating to all participating investigators, any observations reportable under the DFCI IRB Reporting Requirements. Participating Intuitions will review and submit to their IRB according to their institutional policies and procedures.

3.10.2 Guidelines for Processing IND Safety Reports

The DF/HCC Sponsor will review all IND Safety Reports and ensure that all IND Safety Reports are distributed to the Participating Institutions. Participating Institutions will review/submit to their IRB according to their intuitional policies and procedures.

3.11 Data Management

DF/HCC CTRIO develops case report forms (CRF/eCRFs), for use with the protocol. These forms are designed to collect data for the study. DF/HCC CTRIO provides a web based training for all eCRF users.

3.11.1 Data Forms Review

Data submissions are monitored for timeliness and completeness of submission.

If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC Office of Data Quality, Coordinating Center or designee. Responses to all queries should be completed and submitted within 14 calendar days. Responses may be returned within the electronic data capture (eDC) system.

If study forms are not submitted on schedule, the Participating Institution will periodically receive a Missing Form Report from the Coordinating Center noting the missing forms. Required timelines for data submission based on form type are defined in protocol section 11.2.

4.0 REQUISITIONING INVESTIGATIONAL DRUG

Participating Institutions will order their own investigational agent (Pembrolizumab) directly from Merck using the Drug Supply Request Form. Please allow for 3 weeks for drug to arrive after the order is submitted. The Participating Institution will ensure that the pharmacy will be able to receive and store the agent according to state and federal guidelines. The local IRB should be kept informed of who will supply the agent (i.e., Merck pharmaceuticals Inc.) so that any regulatory responsibilities can be met in a timely fashion.

5.0 MONITORING: QUALITY CONTROL

Monitoring and oversight of a clinical trial are federally mandated for all IND held trials. This quality control process for a clinical trial requires verification of protocol compliance and data accuracy and the protection of the rights and welfare of participants. The Coordinating Center, with the aid of the DF/HCC Office of Data Quality provides quality control oversight for the protocol.

5.1 Ongoing Monitoring of Protocol Compliance

The Participating Institutions may be required to submit participant source documents to the Coordinating Center for monitoring. Participating Institutions may also be subject to on-site monitoring conducted by the Coordinating Center.

The Coordinating Center will implement ongoing monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring practices may include but are not limited to

source data verification, and review and analysis of the following: eligibility requirements, informed consent procedures, adverse events and all associated documentation, review of study drug administration/treatment, regulatory files, protocol departures reporting, pharmacy records, response assessments, and data management.

Remote monitoring of participant eligibility, human subject's protection via the initial informed consent process, and screening evaluation completion will occur via a two stage process.

- Prior to registering each participant, a Coordinating Center Specialist will review the source documentation provided in the enrollment packet (see Section B 3.7.1) to confirm, (a) that based on all objective measurements (lab tests; pathology report) that the prospective participant is eligible, (b) that the objective measurements were performed per protocol within the appropriate protocol-defined windows, (c) that the prospective participant does not have concomitant medication that precludes eligibility, and, if documentation is provided, (d) that the consenting process was adequate/adequately documented, (e) that the participant met criteria for eligibility. Furthermore, using the Eligibility Screening Worksheet, the Specialist will verify that the investigator has indicated that he/she has reviewed and confirmed as "eligible" the prospective participant
- The Specialist will review the second set of participant-specific source documents provided by study teams (see Section B 3.7.1) to confirm that (a) all screening and baseline assessments were completed per protocol, including AE assessment, and documented appropriately, (b) that all eligibility criteria were met and appropriately documented, and, if not previously reviewed, (c) that the consenting process was adequate/adequately documented. The timeline for this review will be based on the experience with the study team, and the study team's experience with the protocol.

Interim monitoring visits will occur on the following schedule:

- Once a site has registered a participant, up until all participants (and planned participants) have discontinued taking study agent (may be in follow-up), interim monitoring visits will occur at least twice per year, alternating between on-site visits and virtual monitoring visits. The first on-site interim monitoring visit will occur approximately two months after the registration of the site's first participant.
- Once a site is closed to accrual and all participants have discontinued study agent, interim monitoring visits will occur virtually, and on-site as needed, at least annually until study completion.

On-site monitoring visits will focus on reviewing some or all of the following:

- Adverse events and altered results
- Response assessment including measurements and clinical assessments
- Study drug administration and accountability, to include a visit to the pharmacy
- Concomitant medications
- Re-consenting

- Presence of key documents: original consent, eligibility and screening source information, registration confirmation, off-treatment form, off-study form, transfer of samples
- Reason off treatment and reason off study
- Regulatory binder: accessibility, organization, random sampling for relevant documents and correspondence with the trial master file.
- Visit with the site's principal investigator:
    - Review of site's accrual rate and, when possible, sign-off on the screening/enrollment log to date
    - Review of deviations and violations, and, when possible, sign-off on the deviation/violation log
    - General study progress

Remote monitoring visits will use the trial master file and the electronic data capture system to assess some or all of the following:

- Timeliness of data completion (at the time of the INTERIM MONITORING VISIT and history since last report)
- Attendance at teleconferences since last report
- Completion of study procedures per protocol (procedures complete and within windows)
- Agreement between recorded results and the AE log.
- Analysis of data for any events that met criteria for Reportable Adverse Events, dose holds, dose reductions, or discontinuation of treatment.
- Review the eDC for any Reportable Adverse Events, holds, dose reductions, and discontinuation of treatment to ensure the justification and follow-up are sufficiently documented in the eDC.
- Agreement between drug accountability records and drug administration
- Agreement and completion of the trial master file

Timely transfer of copies of regulatory documents including pharmacy records. The Coordinating Center is mandated to maintain a Trial Master File, which is a copy of the site's regulatory binder.

- Regulatory documents should be sent electronically to the Coordinating Center in lieu of being collected at the time of the monitoring visit; they should be sent upon receipt/creation to the Coordinating Center, and not wait until time of request.
- Original forms (i.e. 1572s, protocol receipts etc.) should be maintained with the site's regulatory binder until requested by the Coordinating Center. When possible, the Coordinating Center will provide a stamped certified copy of the original for the site's regulatory binder.
- Special consideration should be placed on the timely transfer of pharmacy regulatory binder documents, since the Coordinating Center is responsible for tracking all study agent. Copies of DARs and shipping receipts should be provided to the Coordinating

Center via electronic submission on request. The Coordinating Center is to be copied on all drug requests.

Regular all-sites teleconferences to occur at least monthly, bi-weekly when needed. During the teleconferences, sites will be expected to convey the following information:

- Updates on participants taking agent: holds, dose reductions, significant events, how participant is doing, whether or not underwent re-consenting
- Protocol status – which version is being used, and the status of any amendments
- Any Reportable Adverse Events or Deviations/violations that have yet to be communicated to the sponsor team (informing the sponsor should not wait for the call, and the call does not supplant communicating the events via the regular email methods of communication).
- Review of prospective participants

If sites are not able to have a representative participant, they should email this information.

During the teleconferences, the Coordinating Center Specialist will provide the following information at least monthly:

- Accrual/enrollment updates
- Pending amendments
- Safety reports circulated or to be circulated
- ODQ-generated numbers and percentage of missing of missing forms, number of open queries with date of oldest open query, and, for participants on treatment, the date of their last study agent form
- To be updated at least every three months: for participants in follow up, the date of their last follow-up.
- Review of new deviations, violations
- Review of updates to SAEs / new SAEs

During the teleconferences, the Coordinating Center Specialist will provide the following information at least monthly:

- Accrual/enrollment updates
- Pending amendments
- Safety reports circulated or to be circulated
- ODQ-generated numbers and percentage of missing of missing forms, number of open queries with date of oldest open query, and, for participants on treatment, the date of their last study agent form
- To be updated at least every three months: for participants in follow up, the date of their last follow-up.
- Updates (newly discovered events) of deviations, violations
- Updates of Reportable Adverse Events

Monthly circulation of Central Logs and Missing Forms Reports. Approximately

monthly, and usually at the time of the teleconferences, the Coordinating Center Specialist will circulate the updates to the Central Screening Enrollment Log, Central Deviation/violation log, Central Reportable Adverse Event Log, and ODQ generated Missing Forms Reports.

Initial training. To aid with protocol compliance, the Coordinating Center will provide a teleconference site initiation visit (approximately 3 hours) and operations manual (as needed) prior to activation of the study at each site. In addition, the Coordinating Center will provide an overview of the eCRFs with appropriate study team members, after each site has registered their first participant.

The Coordinating Center will be available to all sites' study team members for resolving questions concerns and facilitating compliance.

Because of limited on-site monitoring visits, participating sites will be required to submit (either electronically or via paper) requested source documents to the Coordinating Center for source verification.

5.2 Evaluation of Participating Institution Performance

5.2.1 Monitoring Reports

The DF/HCC Sponsor will be provided with all monitoring reports to ensure protocol compliance. The DF/HCC Sponsor may increase the monitoring activities at Participating Institutions that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations.

5.3 Accrual Monitoring

Prior to extending a protocol to an external site, the DF/HCC Sponsor will establish accrual requirements for each participating institution. Accrual will be monitored for each participating institution by the DF/HCC Sponsor or designee. Sites that are not meeting their accrual expectations may be subject to termination.

Participating Institutions accrual requirement of 3 participants per site annually will be implemented.

6.0 AUDITING: QUALITY ASSURANCE

Auditing is a method of Quality Assurance and involves the systematic and independent examination of all trial related activities and documents. Audits determine if evaluated activities were appropriately conducted and whether data was generated, recorded and analyzed, and accurately reported per the protocol, applicable Standard Operating Procedures (SOPs), and the Code of Federal Regulations (CFR).

6.1 Audit Plan: DF/HCC Sponsored Trials

All Participating Institutions are subject to audit by the DF/HCC Office of Data Quality (ODQ). Typically, approximately 3-4 participants would be audited at the site over a 2 day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

6.2 Audit Notifications

It is the Participating Institution's responsibility to notify the Coordinating Center of all scheduled audit dates (internal or NCI) and re-audit dates (if applicable), which involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

6.3 Audit Reports

The DF/HCC Sponsor will review all final audit reports and corrective action plans, if applicable. The Coordinating Center, must forward any reports to the DF/HCC ODQ per DF/HCC policy for review by the DF/HCC Audit Committee. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

6.4 Participating Institution Performance

The DF/HCC Sponsor and DFCI IRB are charged with considering the totality of an institution's performance in considering institutional participation in the DF/HCC Multi-Center protocol.

Participating Institutions that fail to meet the performance goals of accrual, submission of timely and accurate data, adherence to protocol requirements, and compliance with state and federal regulations, may be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Participating Institutions that fail to demonstrate significant improvement will be considered by the DF/HCC Sponsor for revocation of participation.

A DF/HCC Sponsor and/or the DFCI IRB may terminate a site's participation if it is determined that a site is not fulfilling its responsibilities as described above.

APPENDIX C DFCI REPORTABLE AE COVERSHEET

DFCI Protocol No. 16-225 *Merck Protocol No. 3475-476*

Date:_____ Number of pages including cover sheet: _____ Participant ID/initials _____

To:		**Patrick Wen, M.D. Dana Farber Cancer Institute** EMAIL A COPY OF THIS COVERSHEET AND THE TO NeuroOnc_SAE@dfci.harvard.edu WITH THE WORDS "16-225 SAE" IN THE EMAIL'S SUBJECT LINE.
		Merck Global Safety Attn: Worldwide Product Safety; FAX 215 993-1220

From:	Institution:
Phone No.:	Fax No.:

Reporting Investigator:
Event:

Date Event Met Reporting Criteria (as defined in protocol): ____/_____/_____

Type of Report: ☐ Initial ☐ Follow-up

Toxicity Grade: ☐ G1/mild ☐ G2/moderate ☐ G3/severe ☐ G4/life threatening ☐ G5

Historical/Known Correlation to **Pembrolizumab**: ☐ Expected ☐ Unexpected

Attribution to **Pembrolizumab**: ☐ Unrelated ☐ Unlikely ☐ Possible ☐ Probable ☐ Definite

Meets Definition of Serious AE: ☐ Serious ☐ Non-serious

Signature of Reporting Investigator: _____Date:_____